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(57) Abstract

The present invention relates to nucleotide sequences of vertebrate deltex genes, and amino acid sequences of the encoded vertebrate Deltex proteins. The invention further relates to fragments and other derivatives, and analogs, of vertebrate Deltex proteins, as well as antibodies thereto. Nucleic acids encoding such fragments or derivatives are also within the scope of the invention. Production of the foregoing proteins and derivatives, e.g., by recombinant methods is provided. In a specific embodiment, the invention relates to human deltex nucleic acids and proteins. The present invention also relates to therapeutic and diagnostic methods and compositions based on vertebrate Deltex proteins, nucleic acids, and antibodies. The invention also provides methods of inactivating Notch function in a cell, methods of identifying a compound that inhibits or reduces the binding of a vertebrate Deltex protein to a Notch protein, and methods of expanding non-terminally differentiated cells.

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VERTEBRATE DELTEX PROTEINS, NUCLEIC ACIDS, AND ANTIBODIES, AND RELATED METHODS AND COMPOSITIONS

1. INTRODUCTION

The present invention relates to vertebrate *deltex* genes and their encoded protein products, as well as derivatives and analogs thereof. The invention further relates to production of vertebrate Deltex proteins, derivatives and antibodies. Related therapeutic compositions and methods of therapy and diagnosis are also provided.

2. BACKGROUND OF THE INVENTION

In *Drosophila melanogaster*, the so called "*Notch* group" of genes has been implicated in events crucial for the correct developmental choices of a wide variety of precursor cells (for review, see Fortini and Artavanis-Tsakonas, 1993. Cell 75:1245-1247; Artavanis-Tsakonas and Simpson, 1991, Trends Genet. 7:403-408). The accumulated genetic and molecular studies suggest that these genes encode elements of a cell communication mechanism which includes cell surface, cytoplasmic, and nuclear components.

Very little is known about the mechanisms underlying cell fate choices in higher organisms such as vertebrates; a knowledge of such mechanisms could provide insights into pathologies associated with abnormal differentiation events. Thus, a need exists in the art to obtain and characterize the human members of the "Notch group" of genes, including *deltex*, since these genes appear to play crucial roles in the determination of cell fate.

Numerous developmental genetic studies in recent years have shown that the *Notch* locus plays a central role in regulative events influencing cell fate decisions in *Drosophila* in a very broad spectrum of developing tissues (reviewed in Artavanis-Tsakonas and Simpson, 1991, Trends Genet. 7:403-408; and in Artavanis-Tsakonas et al., 1991, Ann. Rev. Cell Biol. 7:427-452). This pleiotropy of *Notch* function is revealed by mutations affecting all stages of development and a variety of tissues (*e.g.*, Welshons, 1965. Science 150:1122-1229; Welshons, 1971, Genetics 68:259-268; Shellenbarger and Mohler, 1978, Dev Biol. 62:432-446). A dramatic illustration of *Notch* function is seen in the development of the embryonic nervous system, whereby loss of function mutations cause the misrouting of epithelial precursor cells into a neural developmental pathway and result in what has been

termed a 'neurogenic' phenotype (Poulson, 1937, Proc. Natl. Acad. Sci. USA, 23:133-137; Lehman et al., 1983, Roux's Arch. Dev. Biol. 192:62-74).

In attempts to understand the molecular contexts by which the Notch protein communicates signals from the cell surface to the nucleus to effect changes in cell fate, genetic means have been used to identify loci that interact phenotypically with various *Notch* alleles. These genetic studies led to the definition of a small group of interacting loci, which has been operationally termed the 'Notch group' (Artavanis-Tsakonas and Simpson, 1991, Trends Genet. 7:403-408). The other members of the *Notch* group are *deltex* (Xu and Artavanis-Tsakonas, 1991, Genetics 126:665-677), *Enhancer of (split)* [*E(spl)*] (Knust et al., 1987, EMBO J. 6:4113-4123; Hartley et al., 1988, Cell 55:785-795; Preiss et al., 1988, EMBO J. 7:3917-3927; Klambt et al., 1989, EMBO J. 8:203-210), and *mastermind* (*mam*) (Smoller et al., 1990, Genes Dev. 4:1688-1700). *mastermind*, *Hairless (H)*, the *Enhancer of (split)*, and *Suppressor of Hairless (Su(H))* encode nuclear proteins (Smoller et al., 1990, Genes Dev. 4:1688-1700; Bang et al., 1992 Genes Dev. 6:1752-1769; Maier et al., 1992, Mech Dev. 38:143-156; Delidakis et al., 1991, Genetics 129:803-823; Schrons et al., 1992, Genetics 132:481-503; Furukawa et al., 1991, J. Biol Chem. 266:23334-23340; Furukawa et al, 1992, Cell 69:1191-1197; Schweisguth et al., 1992, Cell 69:, 1199-1212). *deltex* mutations suppress the pupal lethality conferred by certain heteroallelic combinations of the *Abruptex* class of *Notch* alleles (Xu et al., 1990, Genes Dev. 4:464-475). From this same genetic screen, the genes *Delta* and *mastermind* were also identified, both of which belong to the same 'neurogenic' class of genes as *Notch* because of the similar mutant phenotypes they produce. Moreover, subsequent analysis has shown that alleles of *deltex* exhibit genetic interactions with those of *Delta*, *mastermind*, *Hairless*, and *Su(H)*, a further suggestion of functional links among these loci (Xu and Artavanis-Tsakonas, 1990, Genetics 126:665-677).

The manner by which *Notch* is thought to influence determinative events is indirect, that is, not through the direct specification of cellular fates. Instead, recent experimental studies (Coffman et al, 1993, Cell 73:659-671; Fortini et al, Nature, in press) indicate that *Notch* activity delays differentiation, and in this manner renders precursor cells competent to receive and/or interpret any number of specific developmental cues (Cagan and Ready, 1989, Genes Dev. 3:1099-1112). In loss of function mutants, this inhibition is lost and cells assume default pathways of differentiation. For example, during the development

of the *Drosophila* nervous system, cells that normally would become epidermis instead adopt a neural fate in the absence of *Notch* function. However, a salient feature of *Notch* activity is its pleiotropy. *Notch* is required for the proper specification of many other cell types, including those of the compound eye (Cagan and Ready, 1989, Genes Dev. 3:1099-1112), ovary (Ruohola et al., 1991, Cell 66:433-449; Xu et al., 1992, Development 115:913-922), and mesoderm (Corbin et al., 1991, Cell 67:311-323). Similarly, the widespread expression patterns exhibited by vertebrate *Notch* cognates suggest also a broad-based functional role in these species (Coffman et al, 1993, Cell 73:659-671; Coffman et al., 1990, Science 249:1438-1441; Weinmaster et al., 1991, Development 113:199-205; Weinmaster et al., 1992, Development 116:931-941; Kopan and Weintraub, 1993, J. Cell Biol. 121:631-641; Franco del Amo et al., 1992, Development 115:737-744; Ellisen et al., 1991, Cell 66:649-661; Stifani et al., 1992, Nature Genetics 2:119-127).

Notch homologs have been isolated from a variety of vertebrate species and have been shown to be remarkably similar to their *Drosophila* counterpart in terms of structure, expression pattern and ligand binding properties (Rebay et al., 1991, Cell 67:687-699; Coffman et al., 1990, Science 249:1438-1441; Ellisen et al, 1991, Cell 66:649-661; Weinmaster et al., 1991, Development 113:199-205). Two human *Notch* homologs have been isolated (PCT Publication No. WO 92/19737 dated November 12, 1992), termed hN and TAN-1. A human *Notch* (*TAN-1*) malfunction has been associated with a lymphatic cancer (Ellisen et al., 1991, Cell 66:649-661).

Notch encodes a large, structurally-complex transmembrane protein, consistent with an involvement in cell-cell communication (Wharton et al., 1985, Cell 43:567-581; Kidd et al., 1986, Mol. Cell. Biol. 6:3094-3108). *Notch* has an extracellular domain containing 36 tandem EGF-like repeats and 3 *Notch/lin12* repeats. The intracellular domain bears several common structural motifs including 6 *cdc10/SW16/ankyrin* repeats ("ANK" repeats) Lux et al., 1990, Nature 344:36-42; Breeden and Nasmyth, 1987, Nature 329:651-654; Michaely and Bennett, 1992, Trends Cell Biol. 2:127-129; Blank et al., 1992, Trends Biochem. Sci. 17:135-140; Bennett, 1992, J. Biol. Chem. 267:8703-8706)), a polyglutamine stretch known as 'opa', and a PEST motif (Stifani et al., 1992, Nature Genetics 2:119-127). The remarkable degree to which these motifs have been conserved in homologs isolated from mice (Weinmaster et al., 1991, Development 113:199-205; Weinmaster et al., 1992, Development 116:931-941; Kopan and Weintraub, 1993, J. Cell

Biol. 121:631-641), rats (Kopan and Weintraub, 1993, J. Cell Biol. 121:631-641; Franco del Amo et al., 1993, Genomics 15:259-264), humans (Ellisen et al., 1991, Cell 66:649-661; Stifani et al., 1992, Nature Genetics 2:119-127; PCT Publication No. WO 92/19737 dated November 12, 1992), and *Xenopus* (Coffman et al, 1993, Cell 73:659-671; Coffman et al., 1990, Science 249:1438-1441) implies that they will have a common biochemical mode of action. In particular, ANK repeats, which constitute the most conserved region (~70% amino acid identity) between Notch and its vertebrate counterparts (Stifani et al., 1992, Nature Genetics 2:119-127), are thought to mediate protein-protein interactions among diverse groups of proteins, including those involved in signal transduction processes and cytoskeletal interactions (Lux et al., 1990, Nature 344:36-42; Breeden and Nasmyth, 1987, Nature 329:651-654; Michaely and Bennett, 1992, Trends Cell Biol. 2:127-129; Blank et al., 1992, Trends Biochem. Sci. 17:135-140; Bennett, 1992, J. Biol. Chem. 267:8703-8706). Indeed, Rebay et al. (1993, Cell 74:319-329) have recently demonstrated that the ANK repeats are crucial for Notch-mediated signaling events. Both EGF-like repeats and ankyrin motifs are found in a variety of proteins known to interact with other protein molecules. Indeed, evidence has shown a direct interaction between Notch and the products of the *Delta* and *Serrate* loci, which also encode transmembrane proteins containing EGF-like repeats (Fehon et al., 1990, Cell 61:523-534; Rebay et al., 1991, Cell 67:687-699).

In *Drosophila*, it has been demonstrated that dominant 'activated' phenotypes result from *in vivo* overexpression of a Notch protein lacking most extracellular, ligand-binding sequences, while 'dominant-negative' phenotypes result from overexpression of a protein lacking most intracellular sequences (Rebay et al., 1993, Cell 74:319-329).

In *Drosophila*, Deltex has been demonstrated to play a critical role in development and other physiological processes, in particular, in the signaling pathway of Notch which is involved in cell fate (differentiation) determination. We have demonstrated through expression studies conducted in cultured *Drosophila* cells, in yeast, and in the imaginal wing disc that *Drosophila* Deltex mediates the intracellular portion of the signal transduction cascade involved in Notch function (Diederich et al., 1994, Development 120:473-481). These studies show that *Drosophila* Deltex is localized within the cytoplasm, that it is a protein of unique sequence, that it displays homotypic interactions, and that it directly physically interacts with the *Drosophila* Notch intracellular ANK repeats.

Additionally, we have demonstrated that *Drosophila* Deltex directly interacts with the ANK repeats of human Notch.

The ANK repeat motif is shared by many proteins and has been implicated in protein-protein interactions (Lux et al., 1990, Nature 344:36-42, Thompson et al., 1991, Science 253:762-768, reviewed in Bennett, 1992, J. Biol. Chem. 267:8703-8706, Blank et al., 1992, Trends Biochem. Sci. 17:135-140, Rebay et al., 1993, Cell 74:319-329). Moreover, an *in vivo* functional analysis of various truncated forms of Notch has implicated these ANK repeats in downstream signaling events and that dominant 'activated' phenotypes result from *in vivo* overexpression of a Notch protein lacking most extracellular, ligand binding sequences, while 'dominant negative' phenotypes result from overexpression of a protein lacking most intracellular sequences (Rebay et al., 1993, Cell, 74:319-329).

Furthermore, *deltex* displays genetic interactions with Notch and Delta, both transmembrane proteins, and with mastermind, a nuclear localized protein (Smoller et al., 1990, Genes Dev. 4:1688-1700). This makes *deltex* the first identified cytoplasmic component of the *Notch* group of interacting loci.

We have also subsequently demonstrated that a fragment mostly composed of the ankyrin repeats of the Notch protein mediate molecular interactions between Notch and the Su(H) protein (Fortini et al., 1994, Cell 79:273-282). The Drosophila *Su(H)* gene encodes a protein of 594 amino acids and binds to the promoters of several viral and cellular genes and interacts directly with a viral transactivator protein termed Epstein-Barr virus nuclear antigen 2 (EBNA2), which enables a virus to subvert the normal program of B cell differentiation (Schweisguth, F., et al, 1992, Cell 69: 1199; Furukawa T., et al., 1991, J. Biol. Chem. 266:23334). Genetic and molecular studies suggest that Deltex and Delta may act in concert to multimerize Notch proteins and to interfere with the cytoplasmic retention of Su(H) by Notch, thus activating the Notch signaling pathway (Diederich, J., et al., 1994, Development 120:473; Fortini, M., et al., 1994, Cell, 79:273; Matsuno, K., et al., unpublished, Artavanis-Tsakonas et al., 1995, Science, 268:225-232). This pathway is believed to control nuclear events in order to influence the progression of uncommitted cells to a more differentiated state. Three loci encoding putative nuclear proteins *Hairless*, *Enhancer of split*, and *mastermind*, have been implicated in these nuclear events.

Despite the cloning of a *Drosophila deltex* gene (See PCT Publication WO 95/19770 published July 27, 1995), no vertebrate *deltex* gene had been obtained prior to the present invention.

Citation of a reference herein shall not be construed as an admission that such reference is prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention relates to nucleotide sequences of vertebrate *deltex* genes, and amino acid sequences of the encoded vertebrate Deltex proteins. The invention further relates to fragments and other derivatives, and analogs, of vertebrate Deltex proteins, as well as antibodies thereto. Nucleic acids encoding such fragments or derivatives are also within the scope of the invention. Production of the foregoing proteins and derivatives, *e.g.*, by recombinant methods, is provided.

In a specific embodiment, the invention relates to human *deltex* nucleic acids and proteins.

In another specific embodiment, the invention relates to mammalian *deltex* nucleic acids and proteins.

In specific embodiments, the invention relates to vertebrate Deltex protein derivatives and analogs of the invention which are functionally active, or which comprise one or more domains of a vertebrate Deltex protein, including but not limited to the SH3-binding domains, ring-H2-Zinc fingers, domains which mediate binding to Notch or to a Notch derivative containing Notch cdc10/SW16/ankyrin ("ANK") repeats, or any combination of the foregoing.

The present invention also relates to therapeutic and diagnostic methods and compositions based on vertebrate Deltex proteins and nucleic acids. The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: vertebrate Deltex proteins and analogs and derivatives (including fragments) thereof; antibodies thereto; nucleic acids encoding the vertebrate Deltex proteins, analogs, or derivatives; and vertebrate *deltex* antisense nucleic acids. In a preferred embodiment, a Therapeutic of the invention is administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a malignant

state. In other specific embodiments, a Therapeutic of the invention is administered to treat a nervous system disorder or to promote tissue regeneration and repair.

In one embodiment, Therapeutics which antagonize, or inhibit, vertebrate Notch and/or Deltex function (hereinafter "Antagonist Therapeutics") are administered for therapeutic effect. In another embodiment, Therapeutics which promote vertebrate Notch and/or Deltex function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect.

Disorders of cell fate, in particular hyperproliferative (*e.g.*, cancer) or hypoproliferative disorders, involving aberrant or undesirable levels of expression or activity or localization of vertebrate Notch and/or Deltex protein can be diagnosed by detecting such levels, as described more fully *infra*.

In a preferred aspect, a Therapeutic of the invention is a protein consisting of at least a fragment (termed herein "adhesive fragment") of vertebrate Deltex which mediates binding to a Notch protein or a fragment thereof.

The invention also provides methods of inactivating Notch function in a cell, methods of identifying a compound that inhibits or reduces the binding of a vertebrate Deltex protein to a Notch protein, and methods of expanding non-terminally differentiated cells.

4. DESCRIPTION OF THE FIGURES

Figure 1A-F. Nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of *Drosophila deltex* cDNA.

Figure 2A-C. Composite nucleotide sequence (SEQ ID NO:11) derived from the cDNA (nucleotide 1 to 2547), and deduced amino acid sequence (SEQ ID NO:12) of the human *deltex* locus. The predicted amino acid sequence is depicted below the DNA sequence. The symbol: * designates the start of T05200 and $ the end of T05200. Core H and C residues in Ring-H2-zinc finger are shown by underlining. PCR primers hdx-1 to 4 (SEQ ID NO:26), (SEQ ID NO:27), (SEQ ID NO:28), and (SEQ ID NO:29), respectively, are indicated in bold. X and N represent amino acid residues and nucleotides, respectively, not yet determined.

Figure 3. Aligned amino acid sequences of human Deltex

(SEQ ID NO:12) and *Drosophila* Deltex (SEQ ID NO:2) proteins. Those positions at which residues are identical are shaded. Sites in which amino acids are chemically similar are boxed.

Figure 4A-B. Amino acid sequence of *Drosophila* Deltex (SEQ ID NO:2) and designated fragments implicated in protein-protein interactions. Fragments A-D (SEQ ID NOS:13-16, respectively) are shown.

Figure 5. Schematic diagram of Deltex fragments mediating Deltex-Deltex interactions.

Figure 6. Schematic diagram of the Deltex and Notch fragments mediating Deltex-Notch interactions.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to nucleotide sequences of vertebrate *deltex* genes, and amino acid sequences of their encoded Deltex proteins. The invention further relates to fragments and other derivatives, and analogs, of vertebrate Deltex proteins. Nucleic acids encoding such fragments or derivatives are also within the scope of the invention. Production of the foregoing proteins and derivatives, *e.g.*, by recombinant methods, is provided.

In a specific embodiment, the invention relates to a human *deltex* gene and protein.

In a another specific embodiment, the invention relates to a mammalian *deltex* gene and protein.

The invention also relates to vertebrate Deltex protein derivatives and analogs of the invention which are functionally active, *i.e.*, they are capable of displaying one or more known functional activities associated with a full-length (wild-type) vertebrate Deltex protein. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with a vertebrate Deltex protein for binding) to an anti-vertebrate Deltex protein antibody], immunogenicity (ability to generate antibody which binds to a vertebrate Deltex protein), ability to bind (or compete with a vertebrate Deltex protein for binding) to Notch or a second Deltex protein or other proteins or fragments thereof, ability to bind (or compete with a vertebrate Deltex protein for binding) to a receptor or ligand for a vertebrate Deltex protein.

The invention further relates to fragments (and derivatives and analogs thereof) of a vertebrate Deltex protein which comprise one or more domains of a vertebrate Deltex protein (see *infra*), including but not limited to the SH3-binding domains, ring-H2-zinc fingers, domains which mediate binding to Notch (or a derivative thereof containing the Notch ANK repeats) or to a second Deltex molecule or fragment thereof, or any combination of the foregoing.

Antibodies to vertebrate Deltex proteins, their derivatives and analogs, are additionally provided.

Our prior attempts to clone human, zebrafish, and *Xenopus deltex* using *Drosophila deltex* as a probe were unsuccessful. In contrast to such prior failures, the present invention is based on the successful cloning of human *deltex*. As described by way of example below, we have used an innovative methodology to clone the transcription unit corresponding to human *deltex*. As described therein (see Section 6), our results show a significant structural conservation of Deltex in humans, indicative of functional conservation. Moreover, we demonstrate that human Deltex displays direct molecular interaction with both human and *Drosophila* Notch intracellular ANK repeats (see Section 7). Knowledge of the sequence of human *deltex* allows the identification of regions strongly conserved between Drosophila and human *deltex*, and provides a method for readily isolating other vertebrate *deltex* genes by use of such strongly conserved regions (see Sections 5.6 and 8 *infra*).

The vertebrate *deltex* nucleic acid and amino acid sequences and antibodies thereto of the invention can be used for the detection and quantitation of vertebrate *deltex* mRNA and protein, to study expression thereof, to produce vertebrate Deltex proteins, fragments and other derivatives, and analogs thereof, in the study, assay, and manipulation of differentiation and other physiological processes, and are of therapeutic and diagnostic use, as described *infra*. The agonists and antagonists of Deltex function can be used to alter the ability of a cell to differentiate. The vertebrate *deltex* nucleic acids and antibodies can also be used to clone vertebrate *deltex* homologs of other species, as described *infra*. Such vertebrate *deltex* homologs are expected to exhibit significant homology to each other, and encode proteins which exhibit the ability to bind to a Notch protein.

The present invention also relates to therapeutic and diagnostic methods and compositions based on vertebrate Deltex proteins and nucleic acids. The invention provides

for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: vertebrate Deltex proteins and analogs and derivatives (including fragments) thereof; antibodies thereto; nucleic acids encoding the vertebrate Deltex proteins, analogs, or derivatives; and vertebrate *deltex* antisense nucleic acids. In a preferred embodiment, a Therapeutic of the invention is administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a malignant state. In other specific embodiments, a Therapeutic of the invention is administered to treat a nervous system disorder or to promote tissue regeneration and repair.

In one embodiment, Therapeutics which antagonize, or inhibit, Notch and/or vertebrate Deltex function (hereinafter "Antagonist Therapeutics") are administered for therapeutic effect. In another embodiment, Therapeutics which promote Notch and/or vertebrate Deltex function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect.

Disorders of cell fate, in particular hyperproliferative (*e.g.*, cancer) or hypoproliferative disorders, involving aberrant or undesirable levels of expression or activity or localization of Notch and/or vertebrate Deltex protein can be diagnosed by detecting such levels, as described more fully *infra*.

In a preferred aspect, a Therapeutic of the invention is a protein consisting of at least a fragment (termed herein "adhesive fragment") of vertebrate Deltex that mediates binding to a Notch protein, a second Deltex protein, or a fragment of Notch or Deltex.

The invention is illustrated by way of examples *infra* which disclose, *inter alia*, the cloning and sequencing of human *deltex*, and the identification of regions of human Deltex which are predicted to bind to the ANK repeats of Notch, or which are predicted to bind to regions of human Deltex.

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections set forth below.

5.1. ISOLATION OF THE VERTEBRATE *DELTEX* NUCLEIC ACIDS

The invention relates to the nucleotide sequences of vertebrate *deltex* nucleic acids. In specific embodiments, human *deltex* nucleic acids comprise the cDNA sequence shown in Figure 2A-C (SEQ ID NO:11), or the coding region thereof (nucleotide numbers

504-2363), or nucleic acids encoding a human Deltex protein (*e.g.*, having the sequence of SEQ ID NO:12). The invention provides nucleic acids consisting of at least 8 nucleotides (*i.e.*, a hybridizable portion) of a vertebrate *deltex* sequence; in other embodiments, the nucleic acids consist of at least 25 (continuous) nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of a vertebrate *deltex* sequence, or a full-length vertebrate *deltex* coding sequence. The invention also relates to nucleic acids hybridizable to or complementary to the foregoing sequences. In specific aspects, nucleic acids are provided which comprise a sequence complementary to at least 10, 25, 50, 100, or 200 nucleotides or the entire coding region of a vertebrate *deltex* gene. In a specific embodiment, the sequence is naturally occurring.

In other specific embodiments, the invention provides nucleic acids comprising at least 110, 150, or 200 continuous nucleotides of the sequence of SEQ ID NO:11. In other embodiments, the invention provides a nucleic acid comprising the first 25, 50, 100, 150, 200, or 230 amino acids of SEQ ID NO:12.

In a specific embodiment, vertebrate *deltex* nucleic acids comprise those nucleic acids which are substantially homologous to the nucleic acids encoding the amino terminal 180 amino acids (encoded, *e.g.*, by nucleotide numbers 504-1044 of SEQ ID:11) of human *deltex*, or fragments thereof. In one embodiment, the vertebrate *deltex* nucleic acid has at least 50% identity over the corresponding nucleotide sequence of an identically sized human *deltex*. In another embodiment this identity is greater than 55%. In a preferred embodiment, the nucleotide sequence identity of the vertebrate *deltex* is greater than 60%. In a more preferred embodiment this identity is greater than 65%. In a most preferred embodiment, the nucleotide sequence identity of the vertebrate *deltex* is greater than 70% over that of the corresponding nucleotide sequence of identically sized human *deltex*.

In another specific embodiment, vertebrate *deltex* nucleic acids comprise those nucleic acids which are substantially homologous to the nucleic acids encoding the central region amino acids of human *deltex* (*e.g.*, nucleotide numbers 1045-1821 of SEQ ID NO:11) or fragments thereof. In one embodiment, the nucleic acids encoding the central amino acids of the vertebrate Deltex protein has at least 50% nucleotide sequence identity with the corresponding human *deltex* sequence of identical size. In another embodiment this identity is greater than 55%. In a preferred embodiment, this nucleotide sequence identity is

greater than 60%. In a more preferred embodiment this identity is greater than 65%. In a most preferred embodiment, the homology of the nucleic acids encoding the central region amino acids of the vertebrate *deltex* has a nucleotide sequence identity that is greater than 65% over that of the corresponding nucleotide sequence of identically sized human *deltex*.

In another specific embodiment, vertebrate *deltex* nucleic acids comprise those nucleic acids which are substantially homologous to the nucleic acids encoding the 180 carboxy terminal amino acids of human *deltex* (nucleotide numbers 1822-2366), or fragments thereof. In one embodiment, the nucleic acids encoding the carboxy terminal region of the vertebrate Deltex protein has at least 50% nucleotide sequence identity over the corresponding human *deltex* sequence of identical size. In another embodiment this identity is greater than 55%. In a preferred embodiment, this identity is greater than 60%. In a more preferred embodiment this identity is greater than 65%. In a most preferred embodiment, the identity of the nucleotides encoding the amino terminal amino acids of the vertebrate *deltex* is greater than 70% over that of the corresponding nucleotide sequence of identically sized human *deltex*.

In a specific embodiment, a nucleic acid which is hybridizable to a vertebrate *deltex* nucleic acid (*e.g.*, having sequence SEQ ID NO:11), or to a nucleic acid encoding a vertebrate *deltex* derivative, under conditions of low stringency is provided. By way of example and not limitation, procedures using such conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792): Filters containing DNA are pretreated for 6 h at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10^6 cpm ^{32}P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40°C, and then washed for 1.5 h at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 h at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations).

In another specific embodiment, a nucleic acid which is hybridizable to a vertebrate *deltex* nucleic acid under conditions of high stringency is provided. By way of example and not limitation, procedures using such conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 μg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C in prehybridization mixture containing 100 μg/ml denatured salmon sperm DNA and 5-20 X 10^6 cpm of ^{32}P-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography. Other conditions of high stringency which may be used are well known in the art.

Nucleic acids encoding derivatives (*e.g.*, fragments) of vertebrate Deltex proteins (see Section 5.6), and vertebrate *deltex* antisense nucleic acids (see Section 5.11) are additionally provided. As is readily apparent, as used herein, a "nucleic acid encoding a fragment or portion of a vertebrate Deltex protein" shall be construed as referring to a nucleic acid encoding only the recited fragment or portion of the vertebrate Deltex protein and not the other contiguous portions of the vertebrate Deltex protein as a continuous sequence.

Specific embodiments for the cloning of a vertebrate *deltex* gene, *e.g.*, a human *deltex* gene, presented as a particular example but not by way of limitation, follows:

For expression cloning (a technique commonly known in the art), an expression library is obtained or is constructed by methods known in the art. For example, mRNA (*e.g.*, human) is isolated, cDNA is made and ligated into an expression vector (*e.g.*, a bacteriophage derivative) such that it is capable of being expressed by the host cell into which it is then introduced. Various screening assays can then be used to select for the expressed vertebrate Deltex product. In a preferred aspect, anti-human Deltex antibodies can be used to select the recombinant host cell expressing a cloned vertebrate *deltex* gene.

In a specific embodiment, PCR is used to amplify the desired vertebrate *deltex* sequence in a genomic or cDNA library, prior to selection (see, by way of example Section 8, *infra*). Oligonucleotide primers representing known vertebrate *deltex* sequences, preferably regions known to be conserved between *Drosophila* and human, can be used as primers in PCR. The synthetic oligonucleotides may be utilized as primers to amplify by

PCR sequences from a source (RNA or DNA), preferably a cDNA library, of potential interest. PCR can be carried out, *e.g.*, by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp™). The DNA being amplified can include human mRNA or cDNA or genomic DNA. One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to allow for greater or lesser degrees of nucleotide sequence similarity between the known vertebrate *deltex* nucleotide sequence and the nucleic acid homolog being isolated. For cross species hybridization, low stringency conditions are preferred (see *supra*). For same species hybridization, moderately stringent or highly stringent conditions are preferred (see *supra*). After successful amplification of a segment of a vertebrate *deltex* gene homolog, that segment may be molecularly cloned and sequenced, and utilized as a probe to isolate a complete cDNA or genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis, as described *infra*. In a preferred aspect, human genes encoding *Deltex* proteins may be identified in this fashion. Alternatively to selection by hybridization, the PCR-amplified DNA can be inserted into an expression vector for expression cloning as described above.

In the event that it is desired to isolate a vertebrate *deltex* gene by cross-species hybridization (either by direct hybridization to a vertebrate *deltex* probe representing all or a part of a vertebrate *deltex* gene of a different species, or by PCR using oligonucleotide primers derived from the sequence of a vertebrate *deltex* gene of a different species), the desired vertebrate *deltex* gene can be isolated as set forth in Example 8, by screening with a probe, or priming for PCR with an oligonucleotide, containing *deltex* sequences encoding regions highly conserved between human and *Drosophila*. For example, the human Deltex amino acid stretches 414-419 (SEQ ID NO:30), 475-480 (SEQ ID NO:31), 504-511 (SEQ ID NO: 32), 531-539 (SEQ ID NO:33) and 557-564 (SEQ ID NO:34) are conserved in *Drosophila* Deltex amino acid stretches 549-555 (SEQ ID NO:35), 603-608 (SEQ ID NO:36), 632-639 (SEQ ID NO:37), 659-667 (SEQ ID NO:38) and 685-692 (SEQ ID NO:39), respectively. In a preferred embodiment, a pair of oligonucleotides comprising sequences separated by a length in the range from 50-500 nucleotides is used as primers in PCR. The invention envisions the use of nucleic acids encoding conserved

regions of the Deltex protein in combination to isolate the Deltex encoding nucleic acids of other organisms, by use in PCR to amplify the desired sequence or less preferably, without PCR, as a probe in selection by virtue of direct colony hybridization (*e.g.*, Grunstein, M. and Hogness, D., 1975, Proc. Natl. Acad. Sci. U.S.A. 72, 3961).

In the event that it is desired to isolate a *deltex* gene by cross-species hybridization (either by direct hybridization to a *deltex* probe representing all or a part of a *deltex* gene of an evolutionarily distant, different species, or by PCR using oligonucleotide primers derived from the sequence of a *deltex* gene of a different, evolutionarily distant species), the desired *deltex* gene can be isolated by a more gradual method of evolutionary walking via first isolating a *deltex* gene from a more closely related species, identifying the portions of *deltex* which are conserved cross-species, and then screening with a probe or priming for PCR with a nucleic acid containing the conserved sequence. This method, while more cumbersome, is straightforward and can be readily carried out by routine methods. For example, if it is desired to proceed further down the evolutionary tree, one may first isolate a murine *deltex* gene using nucleic acids encoding human Deltex as a probe. A conserved portion of the murine *deltex* sequence is then used to screen or amplify *deltex* in an avian library; a conserved portion of the avian clone is used to screen an amphibian library, a conserved portion of the amphibian clone is used to screen a fish library, etc. If desired, the species to be selected in the next round of screening can be selected from among various species by hybridizing the *deltex* probe to a Southern blot containing genomic DNA from each species, and selecting a species to which hybridization occurs.

The above-methods are not meant to limit the following general description of methods by which clones of vertebrate *deltex* may be obtained.

Any eukaryotic cell can potentially serve as the nucleic acid source for the molecular cloning of the vertebrate *deltex* gene. The DNA may be obtained by standard procedures known in the art from cloned DNA (*e.g.*, a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired human cell (see, for example Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 2d. Ed., Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL

Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from genomic DNA, DNA fragments are generated, some of which will encode the desired gene. The DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNAse in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired gene may be accomplished in a number of ways. For example, if an amount of a portion of a vertebrate *deltex* (of any species) gene or its specific RNA, or a fragment thereof *e.g.*, the adhesive domain, is available and can be purified and labeled, the generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton, W. and Davis, R., 1977, Science 196, 180; Grunstein, M. And Hogness, D., 1975, Proc. Natl. Acad. Sci. U.S.A. 72, 3961). Those DNA fragments with substantial homology to the probe will hybridize. For cross species hybridization, low stringency conditions are preferred (see *supra*). For same species hybridization, moderately stringent conditions are preferred (see *supra*). It is also possible to identify the appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map if such is available. Further selection can be carried out on the basis of the properties of the gene. Alternatively, the presence of the gene may be detected by assays based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, can be selected which produce a protein that, *e.g.*, has similar or identical electrophoretic migration, isoelectric focusing behavior, proteolytic digestion maps, binding to Notch, or antigenic properties as known for vertebrate Deltex. If an antibody to vertebrate Deltex is available, the vertebrate Deltex protein may be identified by binding of labeled antibody to the putatively vertebrate Deltex synthesizing clones, in an ELISA (enzyme-linked immunosorbent assay)-type procedure.

The vertebrate *deltex* gene can also be identified by mRNA selection by nucleic acid hybridization followed by *in vitro* translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridization. Such DNA fragments may represent available, purified vertebrate *deltex* DNA of another species (*e.g.*, human). Immunoprecipitation analysis or functional assays (*e.g.*, ability to bind Notch) of the *in vitro* translation products of the isolated products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments that contain the desired sequences. In addition, specific mRNAs may be selected by adsorption of polysomes isolated from cells to immobilized antibodies specifically directed against vertebrate Deltex protein. A radiolabelled vertebrate *deltex* cDNA can be synthesized using the selected mRNA (from the adsorbed polysomes) as a template. The radiolabelled mRNA or cDNA may then be used as a probe to identify the vertebrate *deltex* DNA fragments from among other genomic DNA fragments.

Alternatives to isolating the vertebrate *deltex* genomic DNA include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or making cDNA to the mRNA which encodes the vertebrate *deltex* gene. For example, RNA for cDNA cloning of the vertebrate *deltex* gene can be isolated from cells which express vertebrate Deltex. Other methods are possible and within the scope of the invention.

The identified and isolated gene can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as PBR322, pUC, or Bluescript (Stratagene) plasmid derivatives. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and vertebrate *deltex* gene may be modified by homopolymeric tailing. Recombinant molecules can be introduced

into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the desired gene, for example, by size fractionization, can be done before insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated vertebrate *deltex* gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The vertebrate *deltex* sequences provided by the instant invention include those nucleotide sequences encoding substantially the same amino acid sequences as found in native vertebrate Deltex protein, and those encoded amino acid sequences with functionally equivalent amino acids, all as described in Section 5.6 *infra* for vertebrate Deltex derivatives.

5.2. EXPRESSION OF VERTEBRATE *DELTEX* NUCLEIC ACIDS

The nucleic acid coding for a vertebrate Deltex protein or a functionally active fragment or other derivative thereof can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native vertebrate *deltex* gene and/or its flanking regions. A variety of host-vector systems may be utilized to express the protein-coding sequence. These include but are not limited to vertebrate cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used. In a specific embodiment, a molecule comprising a portion of a vertebrate *deltex* gene which

encodes a protein that binds to Notch or to a molecule comprising the Notch ANK repeats is expressed. In another embodiment, a molecule comprising a portion of a vertebrate *deltex* gene which encodes a protein that binds to a fragment of a Deltex protein is expressed. In other specific embodiments, mammalian *deltex* gene is expressed, or a sequence encoding a functionally active portion of mammalian Deltex. In other specific embodiments, the human *deltex* gene is expressed, or a sequence encoding a functionally active portion of human Deltex. In a specific embodiment, a chimeric protein comprising a Notch-binding domain of a vertebrate Deltex protein is expressed. In other specific embodiments, a full-length vertebrate *deltex* cDNA is expressed, or a sequence encoding a functionally active portion of a vertebrate Deltex protein. In yet another embodiment, a fragment of a vertebrate Deltex protein comprising a domain of the protein, or other derivative, or analog of a vertebrate Deltex protein is expressed.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of a nucleic acid sequence encoding a vertebrate Deltex protein or peptide fragment may be regulated by a second nucleic acid sequence so that the vertebrate Deltex protein or peptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a vertebrate Deltex protein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control vertebrate *deltex* gene expression include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3′ long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase (Villa-Kamaroff et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), *tac* (DeBoer et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25), λP_L, or *trc* promoters; see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; plant expression vectors comprising the nopaline synthetase promoter region or the cauliflower mosaic virus 35S RNA promoter (Gardner et al., 1981, Nucl.

Acids Res. 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Expression vectors containing vertebrate *deltex* gene inserts can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign gene inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted vertebrate *deltex* gene. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to antibiotics, transformation

phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. For example, if the vertebrate *deltex* gene is inserted within the marker gene sequence of the vector, recombinants containing the vertebrate *deltex* insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the vertebrate *deltex* gene product in *in vitro* assay systems, *e.g.*, binding to Notch, binding with antibody.

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (*e.g.*, lambda), and plasmid and cosmid DNA vectors, to name but a few.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered vertebrate Deltex protein may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, phosphorylation, cleavage) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed.

In other specific embodiments, the vertebrate Deltex protein, fragment, analog, or derivative may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer.

Both cDNA and genomic sequences can be cloned and expressed.

In other embodiments, a vertebrate *deltex* cDNA sequence may be chromosomally integrated and expressed. Homologous recombination procedures known in the art may be used.

5.3. IDENTIFICATION AND PURIFICATION OF THE VERTEBRATE *DELTEX* GENE PRODUCTS

In particular aspects, the invention provides amino acid sequences of vertebrate Deltex, preferably human Deltex, and fragments and derivatives thereof which comprise an antigenic determinant (*i.e.*, can be recognized by an antibody) or which are functionally active, as well as nucleic acid sequences encoding the foregoing. "Functionally active" material as used herein refers to that material displaying one or more known functional activities associated with the full-length (wild-type) vertebrate Deltex protein product, *e.g.*, binding to Notch or a portion thereof, binding to another Deltex molecule or portion thereof, binding to any other Deltex ligand, antigenicity (binding to an anti-vertebrate Deltex antibody), immunogenicity (generating anti-Deltex antibody), Notch intracellular signal transduction, etc.

In specific embodiments, the invention provides fragments of a vertebrate Deltex protein consisting of at least 6 amino acids, 10 amino acids, 50 amino acids, or of at least 75 amino acids. In other embodiments, the proteins comprise, or alternatively, consist essentially of; one or more of the SH3-binding domains (*e.g.*, SEQ ID NOS: 17-21 of Table III); one or more ring-H2-zinc finger domains (*e.g.*, SEQ ID NO:25), or a portion which binds to Notch (*e.g.*, comprising the first approximately 230 amino acids of vertebrate Deltex), or any combination of the foregoing, of a vertebrate Deltex protein. Fragments, or proteins comprising fragments, lacking some or all of the foregoing regions of vertebrate Deltex are also provided. Molecules comprising more than one copy of the foregoing regions are also provided. Nucleic acids encoding the foregoing are provided.

Once a recombinant which expresses a vertebrate *deltex* gene sequence is identified, the gene product can be analyzed. This is achieved by assays based on the physical or functional properties of the product, including radioactive labelling of the product followed by analysis by gel electrophoresis, immunoassay, etc. Chemically synthesized proteins, derivatives, and analogs can be similarly analyzed.

Once a vertebrate Deltex protein is identified, it may be isolated and purified by standard methods including chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. The functional properties may be evaluated using any suitable assay (see Section 5.7).

Alternatively, the amino acid sequence of a vertebrate Deltex protein can be deduced from the nucleotide sequence of the chimeric gene contained in the recombinant. Once the amino acid sequence is thus known, the protein can be synthesized by standard chemical methods known in the art (*e.g.*, see Hunkapiller et al., 1984, Nature 310:105-111).

By way of example, the deduced amino acid sequence (SEQ ID NO:12) of a human Deltex protein is presented in Figure 2A-C.

5.4. STRUCTURE OF THE VERTEBRATE *DELTEX* GENES AND PROTEINS

The structure of the vertebrate *deltex* genes and proteins can be analyzed by various methods known in the art.

5.4.1. GENETIC ANALYSIS

The cloned DNA or cDNA corresponding to the vertebrate *deltex* gene can be analyzed by methods including but not limited to Southern hybridization (Southern, 1975, J. Mol. Biol. 98:503-517), Northern hybridization (see *e.g.*, Freeman et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:4094-4098), restriction endonuclease mapping (Maniatis, 1982, Molecular Cloning, A Laboratory, Cold Spring Harbor, New York), and DNA sequence analysis. Polymerase chain reaction (PCR; U.S. Patent Nos. 4,683,202, 4,683,195 and 4,889,818; Gyllenstein et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7652-7656; Ochman et al., 1988, Genetics 120:621-623; Loh et al., 1989, Science 243:217-220) followed by Southern hybridization with a vertebrate *deltex*-specific probe can allow the detection of the vertebrate *deltex* genes in DNA from various cell types. In one embodiment, Southern hybridization can be used to determine the genetic linkage of vertebrate *deltex*. Northern hybridization analysis can be used to determine the expression of the vertebrate *deltex* genes. Various cell types, at various states of development or activity can be tested for vertebrate *deltex* gene expression. The stringency of the hybridization conditions for both Southern

and Northern hybridization can be manipulated to ensure detection of nucleic acids with the desired degree of relatedness to the specific vertebrate *deltex* probe used.

Restriction endonuclease mapping can be used to roughly determine the genetic structure of the vertebrate *deltex* gene. Restriction maps derived by restriction endonuclease cleavage can be confirmed by DNA sequence analysis. Alternatively, restriction maps can be deduced, once the nucleotide sequence is known.

DNA sequence analysis can be performed by any techniques known in the art, including but not limited to the method of Maxam and Gilbert (1980, Meth. Enzymol. 65:499-560), the Sanger dideoxy method (Sanger et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463), the use of T7 DNA polymerase (Tabor and Richardson, U.S. Patent No. 4,795,699; Sequenase, U.S. Biochemical Corp.), or Taq polymerase, or use of an automated DNA sequenator (*e.g.*, Applied Biosystems, Foster City, CA). The cDNA sequence of a human *deltex* gene is shown in Figure 2A-C (SEQ ID NO:11) and is described in Section 6, *infra*.

5.4.2. PROTEIN ANALYSIS

The amino acid sequence of a vertebrate Deltex protein can be derived by deduction from the DNA sequence, or alternatively, by direct sequencing of the protein, *e.g.*, with an automated amino acid sequencer. The amino acid sequence of a representative vertebrate Deltex protein comprises the sequence substantially as depicted in Figure 2A-C (SEQ ID NO:12), and detailed in Section 6, *infra*.

The vertebrate Deltex protein sequence can be further characterized by a hydrophilicity analysis (Hopp and Woods, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824). A hydrophilicity profile can be used to identify the hydrophobic and hydrophilic regions of a vertebrate Deltex protein and the corresponding regions of the gene sequence which encode such regions. Hydrophilic regions are predicted to be immunogenic.

Secondary, structural analysis (Chou and Fasman, 1974, Biochemistry 13:222) can also be done, to identify regions of a vertebrate Deltex protein that assume specific secondary structures.

Manipulation, translation, and secondary structure prediction, as well as open reading frame prediction and plotting, can also be accomplished using computer software programs available in the art.

Other methods of structural analysis can also be employed. These include but are not limited to X-ray crystallography (Engstom, 1974, Biochem. Exp. Biol. 11:7-13) and computer modeling (Fletterick and Zoller (eds.), 1986, Computer Graphics and Molecular Modeling, in Current Communications in Molecular Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

5.5. GENERATION OF ANTIBODIES TO VERTEBRATE DELTEX PROTEINS AND DERIVATIVES THEREOF

According to the invention, a vertebrate Deltex protein, its fragments or other derivatives, or analogs thereof, may be used as an immunogen to generate antibodies which recognize such an immunogen. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. In a preferred embodiment, antibodies which specifically bind to vertebrate Deltex proteins are produced. In a more preferred embodiment, an antibody which binds to a vertebrate Deltex protein (*e.g.*, mammalian, preferably human) but does not bind to (full length) *Drosophila* Deltex protein, is produced. In a preferred embodiment, such an antibody is produced by using as immunogen, regions least conserved between *Drosophila melanogaster* and the vertebrate Deltex protein.

In another embodiment, antibodies to a particular domain of a vertebrate Deltex protein are produced. In a specific embodiment, an antibody is produced which binds to a fragment of vertebrate Deltex which binds to Notch; in another embodiment, an antibody binds to a molecule comprising the first 230 amino-terminal amino acids of vertebrate Deltex. In another embodiment the antibody binds to an amino-terminal fragment of vertebrate Deltex containing not more than the first 200 amino acids of vertebrate Deltex. In yet another embodiment, an antibody binds to a fragment of vertebrate Deltex which binds to a second Deltex molecule.

Various procedures known in the art may be used for the production of polyclonal antibodies to a vertebrate Deltex protein or derivative or analog. In a particular embodiment, rabbit polyclonal antibodies to an epitope of the vertebrate Deltex protein having a sequence depicted in Figure 2A-C or a subsequence thereof, can be obtained. For the production of antibody, various host animals can be immunized by injection with a native vertebrate Deltex protein, or a synthetic version, or derivative (*e.g.*, fragment) thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used

to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

In a preferred embodiment, polyclonal or monoclonal antibodies are produced by use of a hydrophilic portion of a vertebrate Deltex peptide (*e.g.*, identified by the procedure of Hopp and Woods (1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824)).

For preparation of monoclonal antibodies directed toward a vertebrate Deltex protein sequence or analog thereof, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) can be used. In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals (PCT Publication No. WO 89/12690 dated December 28, 1989). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030) or by transforming human B cells with EBV virus *in vitro* (Cole et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, pp. 77-96), or by other methods known in the art. In fact, according to the invention, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851-6855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing the genes from a mouse antibody molecule specific for a vertebrate Deltex protein together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention. Non-human antibodies can be humanized by the method of Winter (see U.S. Patent No. 5,225,539).

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce vertebrate Deltex protein-specific single chain antibodies. An additional embodiment of the invention utilizes

the techniques described for the construction of Fab expression libraries (Huse et al., 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for vertebrate Deltex proteins, derivatives, or analogs.

Antibody fragments and other derivatives which contain the idiotype (binding domain) of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.* ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a specific domain of a vertebrate Deltex protein, one may assay generated hybridomas for a product which binds to a vertebrate Deltex fragment containing such domain. For selection of an antibody specific to human Deltex protein(s), one can select on the basis of positive binding to a human Deltex protein and a lack of binding to *Drosophila* Deltex protein.

In a specific embodiment, antibodies specific to a phosphorylated epitope of vertebrate Deltex are produced.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the protein sequences of the invention *e.g.*, for imaging these proteins, measuring levels thereof in appropriate physiological samples, etc. Antibodies to vertebrate Deltex (since it normally colocalizes with Notch) can be used to determine the intracellular distribution of Notch and/or vertebrate Deltex, in diagnostic methods such as described *infra*. The antibodies also have use in immunoassays. In another embodiment of the invention (see *infra*), anti-vertebrate Deltex antibodies and fragments thereof containing the binding domain are Therapeutics.

5.6. VERTEBRATE DELTEX PROTEINS, DERIVATIVES AND ANALOGS

The invention further provides vertebrate Deltex proteins, and derivatives (including but not limited to fragments) and analogs of vertebrate Deltex proteins. Nucleic acids encoding vertebrate Deltex protein derivatives and protein analogs are also provided. In one embodiment, the vertebrate Deltex proteins are encoded by the vertebrate *deltex*

nucleic acids described in Section 5.1 *supra*. In particular aspects, the proteins, derivatives, or analogs are of mouse or rat; agricultural stock such as cow, sheep, horse, goat, pig and the like; pets such as cats, dogs; or other domesticated mammals, or primate Deltex proteins.

The production and use of derivatives and analogs related to vertebrate Deltex are within the scope of the present invention. In a specific embodiment, the derivative or analog is functionally active, *i.e.*, capable of exhibiting one or more functional activities associated with a full-length, wild-type vertebrate Deltex protein.

In particular, vertebrate Deltex derivatives can be made by altering vertebrate *deltex* sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a vertebrate *deltex* gene may be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of vertebrate *deltex* genes which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the vertebrate Deltex derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of a vertebrate Deltex protein including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

In a specific embodiment of the invention, proteins consisting of or comprising a fragment of a vertebrate Deltex protein consisting of at least 10 (continuous) amino acids of the vertebrate Deltex protein is provided. In other embodiments, the

fragment consists of at least 20 or 50 amino acids of the vertebrate Deltex protein. In specific embodiments, such fragments are not larger than 35, 100 or 200 amino acids. Derivatives or analogs of vertebrate Deltex include but are not limited to those peptides which are substantially homologous to human Deltex or fragments thereof.

In a specific embodiment, derivatives or analogs of vertebrate Deltex include those peptides which are substantially homologous to the amino terminal 180 amino acids (1-180) of human Deltex. In one embodiment, the amino terminal region of the vertebrate Deltex protein has at least 30% identity over the amino terminal amino acid sequence of identically sized human Deltex. In another embodiment this identity is greater than 35%. In a preferred embodiment, the amino terminal amino acid identity of the vertebrate Deltex is greater than 45%. In a more preferred embodiment this identity is greater than 55%. In a most preferred embodiment, the homology of the amino terminal amino acids of the vertebrate Deltex is greater than 65% over the corresponding human Deltex amino terminal amino acid sequence of identical size.

In another specific embodiment, derivatives or analogs of vertebrate Deltex include those peptides which are substantially homologous to the central region (amino acids 181-441) of human Deltex, or fragments thereof. In one embodiment, the central region of the vertebrate Deltex protein has at least 30% identity with the corresponding human Deltex sequence of identical size. In another embodiment this identity is greater than 35%. In a preferred embodiment, the amino acid identity of the central region of vertebrate Deltex and human Deltex is greater than 45%. In a more preferred embodiment this identity is greater than 55%. In a most preferred embodiment, the homology of the central amino acids of the vertebrate Deltex to corresponding human Deltex amino acids of identical size is greater than 65%.

Additionally, derivatives or analogs of vertebrate Deltex include but are not limited to those peptides which are substantially homologous to the carboxy terminal amino acids of human Deltex or fragments thereof. In one embodiment, the carboxy terminal region of the vertebrate Deltex protein (the carboxy terminal 180 amino acids) has at least 45% identity over the amino acid sequence of identical size. In another embodiment this identity is greater than 50%. In a preferred embodiment, the amino terminal amino acid identity of the vertebrate Deltex is greater than 55%. In a more preferred embodiment this identity is greater than 60%. In a most preferred embodiment, the homology of the amino terminal amino acids of the vertebrate Deltex is greater than 65%.

In another preferred embodiment, derivatives or analogs of vertebrate Deltex comprise regions conserved between Drosophila and human Deltex (see Section 8).

The vertebrate Deltex protein derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned vertebrate *deltex* gene sequence can be modified by any of numerous strategies known in the art (Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of the gene encoding a derivative or analog of a vertebrate Deltex protein, care should be taken to ensure that the modified gene remains within the same translational reading frame as the vertebrate *deltex* gene, uninterrupted by translational stop signals, in the gene region where the desired vertebrate Deltex activity is encoded.

Additionally, the vertebrate Deltex-encoding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to, *in vitro* site-directed mutagenesis (Hutchinson et al., 1978, J. Biol. Chem 253:6551), use of TAB® linkers (Pharmacia), etc.

Manipulations of the vertebrate *deltex* sequence may also be made at the protein level. Included within the scope of the invention are vertebrate Deltex protein fragments or other derivatives or analogs which are differentially modified during or after translation, *e.g.*, by acetylation, phosphorylation, carboxylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$, acetylation, formylation, oxidation, reduction, etc.

In a preferred aspect, phosphorylation or, alternatively, dephosphorylation is carried out, which can be to various extents, on the purified vertebrate Deltex protein or

derivative thereof. The phosphorylation state of the molecule may be important to its role in intracellular signal transduction of Notch function. Phosphorylation can be carried out by reaction with an appropriate kinase (*e.g.*, possibly cdc2 or CK II). Dephosphorylation can be carried out by reaction with an appropriate phosphatase.

In addition, analogs and derivatives of vertebrate Deltex proteins can be chemically synthesized. For example, a peptide corresponding to a portion of a vertebrate Deltex protein which comprises the desired domain, or which mediates the desired activity *in vitro*, can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the vertebrate Deltex protein sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α-amino isobutyric acid, 4-aminobutyric acid, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, and Nα-methyl amino acids.

In a specific embodiment, the vertebrate Deltex derivative is a chimeric, or fusion, protein comprising a vertebrate Deltex protein or fragment thereof (preferably consisting of at least a domain or motif of the vertebrate Deltex protein, or at least 10 amino acids of the vertebrate Deltex protein) joined at its amino or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein. In one embodiment, such a chimeric protein is produced by recombinant expression of a nucleic acid encoding the protein (comprising a vertebrate Deltex-coding sequence joined in-frame to a coding sequence for a different protein). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer. A specific embodiment relates to a chimeric protein comprising a fragment of a vertebrate Deltex protein which comprises a domain or motif of the vertebrate Deltex protein, *e.g.*, a portion which binds to a Notch protein or to a second Deltex protein, an SH-3 binding domain, a ring-H2-zinc finger domain, etc. In a particular embodiment, a chimeric nucleic acid can be constructed, encoding a fusion protein consisting of a vertebrate Deltex Notch-binding fragment joined to a non-Deltex protein. As another example, and not by way of limitation, a recombinant

molecule can be constructed according to the invention, comprising coding portions of both a vertebrate *deltex* gene and another gene which is a member of the "Notch group." Another specific embodiment relates to a chimeric protein comprising a fragment of a vertebrate Deltex protein of at least six amino acids. Particular examples of the construction and expression of fusion proteins comprising human Deltex or various Notch fragments, are described in Section 7.

Other specific embodiments of derivatives and analogs are described in the subsection below and examples sections *infra*.

5.6.1. DERIVATIVES OF VERTEBRATE DELTEX CONTAINING ONE OR MORE DOMAINS OF THE PROTEIN

In a specific embodiment, the invention provides vertebrate Deltex derivatives and analogs, in particular vertebrate Deltex fragments and derivatives of such fragments, that comprise or consist of one or more domains of the vertebrate Deltex protein, including but not limited to a region which binds to a Notch protein (or a molecule comprising the ANK repeats thereof), a region which binds to a second Deltex protein or portion thereof, an SH3-binding domain, or a ring-H2-zinc finger domain. In specific embodiments, the vertebrate Deltex derivative may lack all or a portion of one or more of the foregoing domains.

In specific embodiments directed to the domains of the human Deltex protein, the aforesaid domains consist of approximately the following amino-acid sequences (see Section 6.1.1 *infra*):

SH3 binding domains: SEQ ID NOS: 17-21

Ring-H2-zinc finger domain: SEQ ID NO:25

Other binding fragments, *e.g.*, smaller than those set forth above, can be identified by routine methods, *e.g.*, by construction of nucleic acids encoding such fragments and assays for binding (*e.g.*, via the interaction trap method described in Section 7 *infra*.

In a specific embodiment, relating to a vertebrate Deltex protein of a species other than human, fragments comprising specific domains of vertebrate Deltex are those comprising domains in the respective vertebrate Deltex protein most homologous to the specific domain of the human Deltex protein.

We have demonstrated that *Drosophila* Deltex binds to human Notch-1 and 2, suggesting evolutionary conservation of biochemical activity between human and *Drosophila* Deltex. We have also demonstrated that human Deltex binds to human Notch-1 and 2, and that human Deltex binds to *Drosophila* Notch. Using the interaction trap system (described *infra*) as our assay we systematically examined, by deletion analysis, the domains of Notch and Deltex which are responsible for protein-protein interactions. Both Deltex-Deltex as well as Deltex-Notch interactions were detected. Deletion constructs encoding various fragments (described below) of *Drosophila* Deltex, *Drosophila* Notch and human Notch were expressed as fusion constructs (LexA or ACT fusions), and assayed.

The sequences of fragments A-D (SEQ ID NOS:13-16, respectively) of *Drosophila* Deltex which were expressed are shown in Fig. 4A-B.

Figure 5 summarizes the Deltex-Deltex interactions we have detected. Fragment A interacts with Fragment A (homotypic interactions). Fragment B interacts with Fragment B (homotypic interactions). Fragment C interacts with Fragment C (homotypic interactions). In addition, we detected interactions between fragments C and B. However, we can only detect the fragment C-B interaction if fragment C is tested as the "bait" (*i.e.*, as the LexA fusion). If Fragment B is the bait, this interaction is not detected. All the other aforesaid interactions occur irrespective of which fragment is used as the bait. Fragment A consists of amino acids 1-303. Fragment B consists of amino acids 306-486. Fragment C consists of amino acids 514-737.

The heterotypic interaction between Notch and Deltex is occurred between the ANK repeat region of Notch and fragment D of Deltex (which is part of fragment A and includes amino acids 1-204). *Drosophila* Notch ANK repeats as well as the ANK repeats of both human Notch proteins (encoded by TAN-1 and hN) were tested in this interaction assay and showed positive binding to fragment D. The following fragments containing the ANK repeat region were used: *Drosophila* Notch amino acids: 1889-2076 (numbering per Wharton et al., 1985, Cell 43:567-581); Human Notch TAN-1 amino acids: 1826-2146; Human Notch hN amino acids: 1772-2093. All displayed interactions with fragment D. Figure 6 summarizes schematically this interaction.

In specific embodiments, vertebrate Deltex regions are provided that are most homologous to *Drosophila* fragment A (SEQ ID NO:13), fragment B (SEQ ID NO:14), fragment C (SEQ ID NO:15), and fragment D (SEQ ID NO:16), shown in Figure 4A-B.

Binding interactions between fragments are indicated by arrows in Figures 5 and 6. Such regions homologous to A-D are predicted also to display the binding interactions shown in Figures 5 and 6. Thus, amino acids 1-237, 238-391, 392-620, and 1-175 of SEQ ID NO:12 correspond to *Drosophila* fragments A-D, respectively. Molecules comprising one or more of the foregoing regions are provided. Accordingly, by way of example, a molecule comprising amino acid numbers 1-237 of SEQ ID NO:12 is predicted to bind the Notch ankyrin repeats.

Also provided are inhibitors (*e.g.*, peptide inhibitors) of the foregoing protein interactions with Notch or with a second Deltex protein.

The ability to bind to a Notch protein or a Deltex protein (or derivative thereof) can be demonstrated by *in vitro* assays such as the interaction trap technique (Section 7, *infra*).

The nucleic acid sequences encoding Notch or vertebrate Deltex proteins or fragments thereof, for use in such assays, can be isolated from porcine, bovine, equine, feline, canine, as well as primate sources and any other mammals in which homologs of known genes can be identified. For example, the Notch protein or portion thereof comprising the ANK repeats which can be expressed and assayed for binding to Deltex or a Deltex derivative can be derived from any of the Notch homologs: human hN, human TAN-1, *Xenopus*, and *Drosophila*.

Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as the aforesaid domains may be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of the vertebrate *deltex* genes which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the vertebrate Deltex proteins, fragments or derivatives thereof, of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of the domains including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence ("conservative" changes).

The derivatives, analogs, and peptides of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level.

Additionally, the nucleic acid sequence can be mutated *in vitro* or *in vivo*; and manipulations of the sequence may also be made at the protein level.

In addition, analogs and peptides can be chemically synthesized.

5.7. *IN VITRO* ASSAYS OF VERTEBRATE DELTEX PROTEINS, DERIVATIVES AND ANALOGS

The functional activity of vertebrate Deltex proteins, derivatives and analogs, can be assayed *in vitro* by various methods.

For example, in one embodiment, where one is assaying for the ability to bind or compete with wild-type vertebrate Deltex for binding to anti-vertebrate Deltex antibody, various immunoassays known in the art can be used, including but not limited to competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labelled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where one is assaying for the ability to mediate binding to Notch or portion thereof (*e.g.*, Notch ankyrin repeats) to a second Deltex protein or portion thereof, one can carry out assays such as that described *infra* in Section 7.

Other methods will be known to the skilled artisan and are within the scope of the invention.

In another embodiment, a method of identifying a molecule that inhibits or reduces the binding of a vertebrate Deltex protein to a Notch protein is provided. In this manner, agonists and antagonists of Deltex can be identified. Such a method comprises the steps of contacting a Notch protein and a vertebrate Delta protein such that binding between the Notch protein and the Deltex protein can occur, in the presence of one or more

molecules which are desired to be tested for the ability to inhibit or reduce binding between the Notch protein and the Deltex protein, and identifying the molecules that inhibit or reduce the binding of the Deltex protein to the Notch protein. Any of various binding assays known in the art can be used to carry out such a method, including but not limited to yeast interaction trap assays, cell culture *in vitro* aggregation assays, and soluble binding assays using purified Notch and Deltex proteins. A specific embodiment is as follows: Cultured cells are cotransfected with plasmid expression constructs that place *Notch* and *deltex* under distinct inducible promoters. Notch expression in these cells is first induced to ensure proper cell surface localization; Deltex expression is then induced. These cells are then aggregated with cells expressing Delta, to produce mutual capping of Notch and Delta at the point of mutual contact (see Singer J., 1992, Science, 255:1671-1677; Fehon et al., 1990, Cell, 61:523-534; Heitzler, et al., 1991, Cell, 64:1083-1092). Under these conditions, Deltex colocalizes with the capped Notch by virtue of its binding to Notch. The cells are then incubated in the presence of one or more molecules (preferably, purified molecules) which are desired to be tested for the ability to inhibit binding between Notch and Deltex. Molecules which inhibit or reduce the binding of Deltex to Notch will result in an increased localization of Deltex throughout the cell cytoplasm. This increased localization can be determined according to methods known in the art (*e.g.*, immunofluorescent staining with antibody to Deltex). The method can also be carried out using derivatives of Notch and Deltex that mediate binding to Deltex and to Notch, respectively.

5.8. THERAPEUTIC USES

The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: vertebrate Deltex proteins and analogs and derivatives (including fragments) thereof (*e.g.*, as described hereinabove); antibodies thereto (as described hereinabove); nucleic acids encoding the vertebrate Deltex proteins, analogs, or derivatives (*e.g.*, as described hereinabove); and vertebrate *deltex* antisense nucleic acids. As stated *supra*, the Antagonist Therapeutics of the invention are those Therapeutics which antagonize, or inhibit, a vertebrate Deltex function and/or Notch function. Such Antagonist Therapeutics are most preferably identified by use of known convenient *in vitro* assays, *e.g.*, based on their ability to inhibit binding of vertebrate Deltex

to another protein (*e.g.*, a Notch protein), or inhibit any known Notch or vertebrate Deltex function as preferably assayed *in vitro* or in cell culture, although genetic assays (*e.g.*, in *Drosophila* or mouse) may also be employed. In a preferred embodiment, the Antagonist Therapeutic is a protein or derivative thereof comprising a functionally active fragment such as a fragment of vertebrate Deltex which mediates binding to Notch, or an antibody thereto. In other specific embodiments, such an Antagonist Therapeutic is a nucleic acid capable of expressing a molecule comprising a fragment of vertebrate Deltex which binds to Notch, or a vertebrate *deltex* antisense nucleic acid (see Section 5.11 herein). It should be noted that preferably, suitable *in vitro* or *in vivo* assays, as described *infra*, should be utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue, since the developmental history of the tissue may determine whether an Antagonist or Agonist Therapeutic is desired.

In another embodiment of the invention, a nucleic acid containing a portion of a vertebrate *deltex* gene is used, as an Antagonist Therapeutic, to promote vertebrate *deltex* inactivation by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

The Agonist Therapeutics of the invention, as described *supra*, promote vertebrate Deltex function. Such Agonist Therapeutics include but are not limited to proteins and derivatives comprising the portions of Notch that mediate binding to vertebrate Deltex, *i.e.*, the ANK repeats, and nucleic acids encoding the foregoing (which can be administered to express their encoded products *in vivo*).

Further descriptions and sources of Therapeutics of the inventions are found in Sections 5.1 through 5.7 herein.

Molecules which retain, or alternatively inhibit, a desired vertebrate Deltex property, *e.g.*, binding to Notch, binding to an intracellular ligand, can be used therapeutically as inducers, or inhibitors, respectively, of such property and its physiological correlates. In a specific embodiment, a peptide (*e.g.*, in the range of 6-50 or 15-25 amino acids; and particularly of about 10, 15, 20 or 25 amino acids) containing the sequence of a portion of vertebrate Deltex which binds to Notch is used to antagonize Notch function. In a specific embodiment, such an Antagonist Therapeutic is used to treat or prevent human or other malignancies associated with increased Notch expression (*e.g.*, cervical cancer, colon cancer, breast cancer, squamous adenocarcinomas (see *infra*)). Derivatives or analogs of

vertebrate Deltex can be tested for the desired activity by procedures known in the art, including but not limited to the assays described in the examples *infra*. For example, molecules comprising Deltex fragments which bind to Notch ANK repeats (see Section 7), can be obtained and selected by expressing deletion mutants of human Deltex (or of a nucleotide sequence of another species and assaying for binding of the expressed product to Notch by any of several methods, such as the interaction trap system described in the Examples Sections *infra*. In one specific embodiment, peptide libraries can be screened to select a peptide with the desired activity; such screening can be carried out by assaying, *e.g.*, for binding to Notch or a molecule containing the Notch ANK repeats.

The Agonist and Antagonist Therapeutics of the invention have therapeutic utility for disorders of cell fate. The Agonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving an absence or decreased (relative to normal, or desired) levels of Notch or vertebrate Deltex function, for example, in patients where Notch or vertebrate Deltex protein is lacking, genetically defective, biologically inactive or underactive, or underexpressed; and (2) in diseases or disorders wherein *in vitro* (or *in vivo*) assays (see *infra*) indicate the utility of vertebrate Deltex agonist administration. The absence or decreased levels in Notch or vertebrate Deltex function can be readily detected, *e.g.*, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for protein levels, structure and/or activity of the expressed Notch or vertebrate Deltex protein. Many methods standard in the art can be thus employed, including but not limited to immunoassays to detect and/or visualize Notch or vertebrate Deltex protein (*e.g.*, Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect Notch or vertebrate Deltex expression by detecting and/or visualizing respectively Notch or vertebrate *deltex* mRNA (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.)

In vitro assays which can be used to determine whether administration of a specific Agonist Therapeutic or Antagonist Therapeutic is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a Therapeutic, and the effect of such Therapeutic upon the tissue sample is observed. In one embodiment, where the patient has a malignancy, a sample of cells from such malignancy is plated out or grown in culture, and the cells are then exposed

to a Therapeutic. A Therapeutic which inhibits survival or growth of the malignant cells (*e.g.*, by promoting terminal differentiation) is selected for therapeutic use *in vivo*. Many assays standard in the art can be used to assess such survival and/or growth; for example, cell proliferation can be assayed by measuring ^{3}H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogenes (*e.g.*, *fos*, *myc*) or cell cycle markers; cell viability can be assessed by trypan blue staining, differentiation can be assessed visually based on changes in morphology, etc. In a specific aspect, the malignant cell cultures are separately exposed to (1) an Agonist Therapeutic, and (2) an Antagonist Therapeutic; the result of the assay can indicate which type of Therapeutic has therapeutic efficacy.

In another embodiment, a Therapeutic is indicated for use which exhibits the desired effect, inhibition or promotion of cell growth, upon a patient cell sample from tissue having or suspected of having a hyper- or hypoproliferative disorder, respectively. Such hyper- or hypoproliferative disorders include but are not limited to those described in Sections 5.8.1 through 5.8.3 *infra*.

In another specific embodiment, a Therapeutic is indicated for use in treating nerve injury or a nervous system degenerative disorder (see Section 5.8.2) which exhibits *in vitro* promotion of nerve regeneration/neurite extension from nerve cells of the affected patient type.

In addition, administration of an Antagonist Therapeutic of the invention is also indicated in diseases or disorders determined or known to involve a Notch or Deltex dominant activated phenotype ("gain of function" mutations.) Administration of an Agonist Therapeutic is indicated in diseases or disorders determined or known to involve a Notch or Deltex dominant negative phenotype ("loss of function" mutations). The functions of various structural domains of the Notch protein have been investigated *in vivo*, by ectopically expressing a series of *Drosophila Notch* deletion mutants under the hsp70 heat-shock promoter, as well as eye-specific promoters (see Rebay et al., 1993, Cell 74:319-329). Two classes of dominant phenotypes were observed, one suggestive of *Notch* loss-of function mutations and the other of *Notch* gain-of-function mutations. Dominant "activated" phenotypes resulted from overexpression of a protein lacking most extracellular sequences, while dominant "negative" phenotypes resulted from overexpression of a protein lacking most intracellular sequences. The results indicated that Notch functions as a

receptor whose extracellular domain mediates ligand-binding, resulting in the transmission of developmental signals by the cytoplasmic domain. The phenotypes observed also suggested that the ANK repeat region within the intracellular domain plays an essential role in Notch mediated signal transduction events (intracellular function). We have shown that *Drosophila* Deltex binds to the Notch ANK repeat region.

In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a patient's disorder, to determine if a Therapeutic has a desired effect upon such cell types.

In another embodiment, cells of a patient tissue sample suspected of being pre-neoplastic are similarly plated out or grown *in vitro*, and exposed to a Therapeutic. The Therapeutic which results in a cell phenotype that is more normal (*i.e.*, less representative of a pre-neoplastic state, neoplastic state, malignant state; or transformed phenotype) is selected for therapeutic use. Many assays standard in the art can be used to assess whether a pre-neoplastic state, neoplastic state, or a transformed or malignant phenotype, is present. For example, characteristics associated with a transformed phenotype (a set of *in vitro* characteristics associated with a tumorigenic ability *in vivo*) include a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton surface protein, etc. (see Luria et al., 1978, *General Virology*, 3d Ed., John Wiley & Sons, New York pp. 436-446).

In other specific embodiments, the *in vitro* assays described *supra* can be carried out using a cell line, rather than a cell sample derived from the specific patient to be treated, in which the cell line is derived from or displays characteristic(s) associated with the malignant, neoplastic or pre-neoplastic disorder desired to be treated or prevented, or is derived from the neural or other cell type upon which an effect is desired, according to the present invention.

In a specific embodiment, an antagonist of Notch and/or Deltex function that can be used as an Antagonist Therapeutic is a molecule comprising a Deltex protein or portion thereof that mediates binding to Notch, covalently bound to a protease or proteolytically active fragment thereof. Such protease preferably is able to cleave a Notch protein. The molecule is preferably a fusion protein (*i.e.*, the covalent bond is a peptide

bond). The Deltex protein is preferably a vertebrate protein, most preferably human. Accordingly, the invention provides a method of targeting or inactivating proteins to which Deltex binds (*e.g.*, Notch) in a cell. According to this method, the molecule comprising the Deltex protein or portion thereof and the protease sequences is produced through chemical or via molecular biological techniques. This molecule (*e.g.*, fusion protein) is introduced into the cell by techniques known in the art (*e.g.*, transfection of the cell with a nucleic acid encoding the molecule such that its expression occurs intracellularly). Inside the cell, the molecule can bind to Notch and/or other Deltex binding partners. Upon such binding, the protease portion of the molecule cleaves the protein to which the molecule is bound, thus inactivating it. For example, a fusion protein containing domain I of human Deltex and the protease thermolysin, when introduced into the cell would bind to and cleave Notch, thereby inactivating the Notch signaling pathway. Molecules which would inactivate protein function *e.g.*, by binding thereto, can be used as an alternative to proteases.

The Antagonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving increased (relative to normal, or desired) levels of Notch or vertebrate Deltex function, for example, where the Notch or vertebrate Deltex protein is overexpressed or overactive; and (2) in diseases or disorders wherein *in vitro* (or *in vivo*) assays indicate the utility of vertebrate Deltex antagonist administration. The increased levels of Notch or vertebrate Deltex function can be readily detected by methods such as those described above, by quantifying protein and/or RNA. *In vitro* assays with cells of patient tissue sample or the appropriate cell line or cell type, to determine therapeutic utility, can be carried out as described above.

5.8.1. MALIGNANCIES

Malignant and pre-neoplastic conditions which can be tested as described *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to those described below in Sections 5.8.1 and 5.9.1.

Malignancies and related disorders, cells of which type can be tested *in vitro* (and/or *in vivo*), and upon observing the appropriate assay result, treated according to the present invention, include but are not limited to those listed in Table 1 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia):

TABLE 1
MALIGNANCIES AND RELATED DISORDERS

Leukemia
- acute leukemia
  - acute lymphocytic leukemia
  - acute myelocytic leukemia
    - myeloblastic
    - promyelocytic
    - myelomonocytic
    - monocytic
    - erythroleukemia
- chronic leukemia
  - chronic myelocytic (granulocytic) leukemia
  - chronic lymphocytic leukemia

Polycythemia vera
Lymphoma
- Hodgkin's disease
- non-Hodgkin's disease

Multiple myeloma
Waldenström's macroglobulinemia
Heavy chain disease
Solid tumors
- sarcomas and carcinomas
  - fibrosarcoma
  - myxosarcoma
  - liposarcoma
  - chondrosarcoma
  - osteogenic sarcoma
  - chordoma
  - angiosarcoma
  - endotheliosarcoma
  - lymphangiosarcoma
  - lymphangioendotheliosarcoma
  - synovioma
  - mesothelioma
  - Ewing's tumor
  - leiomyosarcoma
  - rhabdomyosarcoma
- colon carcinoma
- pancreatic cancer
- breast cancer
- ovarian cancer
- prostate cancer
- squamous cell carcinoma
- basal cell carcinoma

adenocarcinoma
sweat gland carcinoma
sebaceous gland carcinoma
papillary carcinoma
papillary adenocarcinomas
cystadenocarcinoma
medullary carcinoma
bronchogenic carcinoma
renal cell carcinoma
hepatoma
bile duct carcinoma
choriocarcinoma
seminoma
embryonal carcinoma
Wilms' tumor
cervical cancer
testicular tumor
lung carcinoma
small cell lung carcinoma
bladder carcinoma
epithelial carcinoma
glioma
astrocytoma
medulloblastoma
craniopharyngioma
ependymoma
pinealoma
hemangioblastoma
acoustic neuroma
oligodendroglioma
menangioma
melanoma
neuroblastoma
retinoblastoma

In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias) are treated or prevented in epithelial tissues such as those in the cervix, esophagus, and lung.

Malignancies of the colon and cervix can exhibit increased expression of human Notch relative to such non-malignant tissue (see PCT Publication WO 94/07474 published April 14, 1994, incorporated by reference herein in its entirety). Thus, in specific embodiments, malignancies of the colon or cervix are treated or prevented by administering an effective amount of an Antagonist Therapeutic of the invention. The presence of

increased Notch expression in colon, and cervical cancer suggests that many more cancerous and hyperproliferative conditions exhibit upregulated Notch. Thus, in specific embodiments, various cancers, *e.g.*, breast cancer, squamous adenocarcinoma, seminoma, melanoma, and lung cancer, as well as other hyperproliferative disorders, can be treated or prevented by administration of an Antagonist Therapeutic.

5.8.2. NERVOUS SYSTEM DISORDERS

Nervous system disorders, involving cell types which can be tested as described *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human vertebrate patients) according to the invention include but are not limited to the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems:

(i) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;

(ii) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;

(iii) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue;

(iv) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;

(v) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including

but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis;

(vi) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration;

(vii) neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis;

(viii) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and

(ix) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including but not limited to multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons (see also Section 5.8). For example, and not by way of limitation, Therapeutics which elicit any of the following effects may be useful according to the invention:

(i) increased survival time of neurons in culture;

(ii) increased sprouting of neurons in culture or *in vivo*;

(iii) increased production of a neuron-associated molecule in culture or *in vivo*, *e.g.*, choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or

(iv) decreased symptoms of neuron dysfunction *in vivo*.

Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann. Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, *e.g.*, weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron disorders that may be treated according to the invention include but are not limited to disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including but not limited to progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

5.8.3. TISSUE REPAIR AND REGENERATION

In another embodiment of the invention, a Therapeutic of the invention is used for promotion of tissue regeneration and repair, including but not limited to treatment of benign dysproliferative disorders. Specific embodiments are directed to treatment of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes), treatment of keloid (hypertrophic scar) formation (disfiguring of the skin in which the scarring process interferes with normal renewal), psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate determination), and baldness (a condition in which terminally differentiated hair follicles (a tissue rich in Notch) fail to function properly).

Deltex agonists and antagonists can also be used to manipulate the differentiation state of non-terminally differentiated (e.g., stem and progenitor) cells. For example, a stem cell can be exposed to such an agonist to inhibit its differentiation and

achieve expansion of the stem cell population by incubation *in vitro* under growth conditions. Such stem cells have use in transplantation for *in vivo* repopulation of their progeny cells and tissue regeneration. (For methods that can be used in the foregoing, see United States patent application Serial No. 08/537,210 filed September 29, 1995 by Artavanis-Tsakonas et al., entitled "Manipulation of Non-Terminally Differentiated Cells Using the Notch Pathway," which is incorporated by reference herein in its entirety.) For example, a method for the expansion of a precursor cell (*e.g.*, a human stem or progenitor cell) comprises contacting the cell with an amount of a vertebrate (*e.g.*, human) Deltex protein or functionally active portion thereof effective to inhibit differentiation of the cell, and exposing the cell to cell growth conditions such that the cell proliferates. In various embodiments, the precursor cell can be but is not limited to a hematopoietic precursor cell, epithelial precursor cell, kidney precursor cell, neural precursor cell, skin precursor cell, osetoblast precursor cell, chondrocyte precursor cell, liver precursor cell, and muscle cell.

5.9. PROPHYLACTIC USES

5.9.1. MALIGNANCIES

The Therapeutics of the invention can be administered to prevent progression to a neoplastic or malignant state, including but not limited to those disorders listed in Table 1. Such administration is indicated where the Therapeutic is shown in assays, as described *supra*, to have utility for treatment or prevention of such disorder. Such prophylactic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79.) Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell

uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can indicate the desirability of prophylactic/therapeutic administration of a Therapeutic of the invention. As mentioned *supra*, such characteristics of a transformed phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton cell surface protein, etc. (see also *id.*, at pp. 84-90 for characteristics associated with a transformed or malignant phenotype).

In a specific embodiment, leukoplakia, a benign-appearing hyperplastic or dysplastic lesion of the epithelium, or Bowen's disease, a carcinoma *in situ*, are pre-neoplastic lesions indicative of the desirability of prophylactic intervention.

In another embodiment, fibrocystic disease (cystic hyperplasia, mammary dysplasia, particularly adenosis (benign epithelial hyperplasia)) is indicative of the desirability of prophylactic intervention.

In other embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: a chromosomal translocation associated with a malignancy (*e.g.*, the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (a possible forerunner of multiple myeloma), and a first degree kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia

telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; *see* Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 112-113) etc.)

In another specific embodiment, an Antagonist Therapeutic of the invention is administered to a human patient to prevent progression to breast, colon, or cervical cancer.

5.9.2. OTHER DISORDERS

In other embodiments, a Therapeutic of the invention can be administered to prevent a nervous system disorder described in Section 5.8.2, or other disorder (*e.g.*, liver cirrhosis, psoriasis, keloids, baldness) described in Section 5.8.3.

5.10. DEMONSTRATION OF THERAPEUTIC OR PROPHYLACTIC UTILITY

The Therapeutics of the invention can be tested *in vivo* for the desired therapeutic or prophylactic activity. For example, such compounds can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used.

5.11. ANTISENSE REGULATION OF VERTEBRATE DELTEX EXPRESSION

The present invention provides the therapeutic or prophylactic use of nucleic acids of at least six nucleotides that are antisense to a gene or cDNA encoding vertebrate Deltex or a portion thereof. "Antisense" as used herein refers to a nucleic acid capable of hybridizing to a portion of a vertebrate *deltex* RNA (preferably mRNA) by virtue of some sequence complementarity. Such antisense nucleic acids have utility as Antagonist Therapeutics of the invention, and can be used in the treatment or prevention of disorders as described *supra* in Section 5.8 and its subsections.

The antisense nucleic acids of the invention can be oligonucleotides that are double-stranded or single-stranded, RNA or DNA or a modification or derivative thereof, which can be directly administered to a cell, or which can be produced intracellularly by transcription of exogenous, introduced sequences.

In a specific embodiment, the vertebrate *deltex* antisense nucleic acids provided by the instant invention can be used for the treatment of tumors or other disorders, the cells of which tumor type or disorder can be demonstrated (*in vitro* or *in vivo*) to express a vertebrate *deltex* gene or a *Notch* gene. Such demonstration can be by detection of RNA or of protein.

The invention further provides pharmaceutical compositions comprising an effective amount of the vertebrate *deltex* antisense nucleic acids of the invention in a pharmaceutically acceptable carrier, as described *infra* in Section 5.12. Methods for treatment and prevention of disorders (such as those described in Sections 5.8 and 5.9) comprising administering the pharmaceutical compositions of the invention are also provided.

In another embodiment, the invention is directed to methods for inhibiting the expression of a vertebrate *deltex* nucleic acid sequence in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising an antisense vertebrate *deltex* nucleic acid of the invention.

Vertebrate *deltex* antisense nucleic acids and their uses are described in detail below.

5.11.1. VERTEBRATE *DELTEX* ANTISENSE NUCLEIC ACIDS

The vertebrate *deltex* antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides (ranging from 6 to about 50 oligonucleotides). In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO 88/09810, published December 15, 1988) or blood-brain barrier (see, *e.g.*, PCT Publication No. WO 89/10134, published April 25, 1988), hybridization-triggered cleavage agents (see, *e.g.*, Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, Pharm. Res. 5:539-549).

In a preferred aspect of the invention, a vertebrate *deltex* antisense oligonucleotide is provided, preferably of single-stranded DNA. In a most preferred aspect, such an oligonucleotide comprises a sequence antisense to the sequence encoding an SH3-binding domain or a Notch-binding domain of vertebrate *deltex* or zinc finger domain, most preferably, of human *deltex*. The oligonucleotide may be modified at any position on its structure with substituents generally known in the art.

The vertebrate *deltex* antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

In another embodiment, the oligonucleotide comprises at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the oligonucleotide is an α-anomeric oligonucleotide. An α-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641).

The oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.*, by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

In a specific embodiment, the vertebrate *deltex* antisense oligonucleotide comprises catalytic RNA, or a ribozyme (see, *e.g.*, PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al., 1990, Science 247:1222-1225). In another embodiment, the oligonucleotide is a 2'-0-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

In an alternative embodiment, the vertebrate *deltex* antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the vertebrate *deltex* antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding the vertebrate *deltex* antisense RNA can be by any promoter known in the art to act in vertebrate, preferably human, cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner

et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a vertebrate *deltex* gene, preferably a human *deltex* gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded vertebrate *deltex* antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a vertebrate *deltex* RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

5.11.2. THERAPEUTIC UTILITY OF VERTEBRATE *DELTEX* ANTISENSE NUCLEIC ACIDS

The vertebrate *deltex* antisense nucleic acids can be used to treat (or prevent) malignancies or other disorders, of a cell type which has been shown to express vertebrate *deltex* or *Notch*. In specific embodiments, the malignancy is cervical, breast, or colon cancer, or squamous adenocarcinoma. Malignant, neoplastic, and pre-neoplastic cells which can be tested for such expression include but are not limited to those described *supra* in Sections 5.8.1 and 5.9.1. In a preferred embodiment, a single-stranded DNA antisense vertebrate *deltex* oligonucleotide is used.

Malignant (particularly, tumor) cell types which express vertebrate *deltex* or *Notch* RNA can be identified by various methods known in the art. Such methods include but are not limited to hybridization with a vertebrate *deltex* or *Notch*-specific nucleic acid (*e.g.*, by Northern hybridization, dot blot hybridization, *in situ* hybridization), observing the ability of RNA from the cell type to be translated *in vitro* into Notch or vertebrate Deltex, immunoassay, etc. In a preferred aspect, primary tumor tissue from a patient can be assayed for Notch or vertebrate Deltex expression prior to treatment, *e.g.*, by immunocytochemistry or *in situ* hybridization.

Pharmaceutical compositions of the invention (see Section 5.12), comprising an effective amount of a vertebrate *deltex* antisense nucleic acid in a pharmaceutically acceptable carrier, can be administered to a patient having a malignancy which is of a type that expresses *Notch* or vertebrate *deltex* RNA or protein.

The amount of vertebrate *deltex* antisense nucleic acid which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the antisense cytotoxicity of the tumor type to be treated *in vitro*, and then in useful animal model systems prior to testing and use in humans.

In a specific embodiment, pharmaceutical compositions comprising vertebrate *deltex* antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, it may be useful to use such compositions to achieve sustained release of the vertebrate *deltex* antisense nucleic acids. In a specific embodiment, it may be desirable to utilize liposomes targeted via antibodies to specific identifiable tumor antigens (Leonetti et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2448-2451; Renneisen et al., 1990, J. Biol. Chem. 265:16337-16342).

5.12. THERAPEUTIC/PROPHYLACTIC ADMINISTRATION AND COMPOSITIONS

The invention provides methods of treatment (and prophylaxis) by administration to a subject of an effective amount of a Therapeutic of the invention. In a preferred aspect, the Therapeutic is substantially purified. The subject is preferably an animal, including but not limited to animals such as cows, pigs, chickens, etc., and is preferably a mammal, and most preferably human.

Various delivery systems are known and can be used to administer a Therapeutic of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, expression by recombinant cells, receptor mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a Therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be

administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In another embodiment, the Therapeutic can be delivered in a vesicle, in particular a liposome (*see* Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; *see* generally *ibid.*)

In yet another embodiment, the Therapeutic can be delivered in a controlled release system. In one embodiment, a pump may be used (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (*see* Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); *see also* Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, *i.e.*, the brain, thus requiring only a fraction of the

systemic dose (*see*, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment, administration of a Therapeutic into a Notch-expressing cell is accomplished by linkage of the Therapeutic to a Delta (or other toporythmic) protein or portion thereof capable of mediating binding to Notch. Contact of a Notch-expressing cell with the linked Therapeutic results in binding of the linked Therapeutic via its Delta portion to Notch on the surface of the cell, followed by uptake of the linked Therapeutic into the Notch-expressing cell.

In a specific embodiment, the Therapeutic is delivered intracellularly (*e.g.*, by expression from a nucleic acid vector, or by linkage to a Delta protein capable of binding to Notch followed by binding and internalization, or by receptor-mediated or diffusion mechanisms).

In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid Therapeutic can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

In specific embodiments directed to treatment or prevention of particular disorders, preferably the following forms of administration are used:

Disorder	Preferred Forms of Administration
Cervical cancer	Topical
Gastrointestinal cancer	Oral; intravenous
Lung cancer	Inhaled; intravenous

Leukemia	Intravenous; extracorporeal
Metastatic carcinomas	Intravenous; oral
Brain cancer	Targeted; intravenous; intrathecal
Liver cirrhosis	Oral; intravenous
Psoriasis	Topical
Keloids	Topical
Baldness	Topical
Spinal cord injury	Targeted; intravenous; intrathecal
Parkinson's disease	Targeted; intravenous; intrathecal
Motor neuron disease	Targeted; intravenous; intrathecal
Alzheimer's disease	Targeted; intravenous; intrathecal

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate,

etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the Therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. In another preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to mammals. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for

intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

5.13. DIAGNOSTIC UTILITY

Vertebrate Deltex proteins, analogues, derivatives, and subsequences thereof, vertebrate *deltex* nucleic acids (and sequences complementary thereto), anti-vertebrate Deltex antibodies, have uses in diagnostics. Such molecules can be used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders affecting vertebrate Deltex expression, or monitor the treatment thereof. In particular, such an immunoassay is carried out by a method comprising contacting a sample derived from a patient with an anti-vertebrate Deltex antibody under conditions such that immunospecific binding can occur, and detecting or measuring the amount of any immunospecific binding by the antibody. In a specific aspect, such binding of antibody, in tissue sections, preferably in conjunction with binding of anti-Notch can be used to detect aberrant Notch and/or vertebrate Deltex localization or aberrant levels of Notch-vertebrate Deltex colocalization in a disease state. In a specific embodiment, antibody to vertebrate Deltex can be used to assay in a patient tissue or serum sample for the presence of vertebrate Deltex where an aberrant level of vertebrate Deltex is an indication of a diseased condition. Aberrant levels of vertebrate Deltex binding ability in an endogenous Notch protein, or aberrant levels of binding ability to Notch (or other vertebrate Deltex ligand) in an endogenous vertebrate Deltex protein may be indicative of a disorder of cell fate (*e.g.*, cancer, etc.) By "aberrant levels," is meant increased or decreased levels relative to that

present, or a standard level representing that present, in an analogous sample from a subject not having the disorder.

The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few.

Vertebrate *deltex* genes and related nucleic acid sequences and subsequences, including complementary sequences, and other toporythmic gene sequences, can also be used in hybridization assays. Vertebrate *deltex* nucleic acid sequences, or subsequences thereof comprising about at least 8 nucleotides, can be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant changes in vertebrate Deltex expression and/or activity as described *supra*. In particular, such a hybridization assay is carried out by a method comprising contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to vertebrate *deltex* DNA or RNA, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization.

6. EXAMPLE: CLONING AND CHARACTERIZATION OF HUMAN *DELTEX*

As described herein, we have accomplished the isolation and molecular characterization of human *deltex*. We report the cloning and sequencing of human *deltex*. Human *deltex* encodes five putative SH3 domain binding sites and a ring-H2-zinc finger in similar locations to the corresponding motifs found in *Drosophila* Deltex.

6.1. RESULTS

6.1.1. MOLECULAR CLONING OF THE HUMAN DELTEX LOCUS

Human *deltex* was isolated through a combination of computer and biochemical screens. Initially, a human expressed sequence tag database was screened for homology against the amino acid sequence of *Drosophila* Deltex. The critical part of this search involved the assumption that stop codons in a particular reading frame of the database

are the result of sequencing mistakes. Accordingly, stop codons were ignored and the open reading frame was extended in a different frame. The predicted amino acid sequence encoded by the hypothetical open reading frames were then compared with the protein product of the *Drosophila deltex* transcription unit.

We previously identified the *Drosophila deltex* transcription unit by showing via germline-mediated transformation experiments that a genomic fragment containing this transcription unit is capable of complementing most *deltex* mutant defects. Moreover, this genomic fragment rescues the normally lethal genetic interaction that results when flies are doubly mutant for *deltex* and *nd*. Finally, Northern analysis indicates a maternal loading of *deltex* transcripts into the developing oocyte, a finding that is consistent with the maternal effect observed upon embryogenesis in eggs laid by homozygous mutant mothers (Xu and Artavanis-Tsakonas, 1990 Genetics 126:665-677). cDNA clones homologous to the transcription unit were isolated from an embryonic cDNA library, the complete nucleotide sequence (SEQ ID NO:1) and predicted protein product were then determined (SEQ ID NO:2).

Comparison of the amino acid sequence of *Drosophila* Deltex with that predicted for what we deduced to be hypothetical open reading frames in the database identified a sequence:gnl I dbest I 24254 T05200 with significant homology to *Drosophila* Deltex. Within T05200, five conserved stretches of amino acids were found in different reading frames, at residues 7-39 (SEQ ID NO:4), 102-149 (SEQ ID NO:6), 138-245 (SEQ ID NO:8), and 200-310 (SEQ ID NO:10) corresponding to *Drosophila* Deltex residues 545-555 (SEQ ID NO:3), 565-580 (SEQ ID NO:5), 581-616 (SEQ ID NO:7) and 602-638 (SEQ ID NO:9) respectively. These sequences are shown in Table II, identical amino acids are shown in bold.

TABLE II

	AMINO ACID NOS.	
Drosophila Deltex	602-638	VYGEKVGVQPIGSMSWSIISKNLPGHEGQNTIQIVYD
T05200	200-310	IYGEKTGTQPPGKMEFHLIPHSLXFGPDTQTXRIVYD
Drosophila Deltex	565-580	LSRCQHLMHLQCLNGM
T05200	102-149	LGRCGHMYHLLCLVAM
Drosophila Deltex	581-616	IIAQQNEMNKNLFIECPVCGIVYGEKVGNQPIGSMS
T05200	138-245	LVAMYSNGNKDGSLQCPTCKPSMGRRRVRSRLGRWS
Drosophila Deltex	545-555	QPCPMCMEELV
T05200	7-39	EDCTICMERLV

A series of two 5' primers (hdx-1 (SEQ ID NO:26) and hdx-2 (SEQ ID NO:27)) and two 3' primers (hdx-3 (SEQ ID NO:28) and hdx-4 (SEQ ID NO:29)) were synthesized based on the DNA sequence of gnl I dbest I 24254 T05200. PCR reactions were performed using the four different primer combinations and a human fetal brain cDNA library (Invitrogene) as the template. The PCR product was sequenced and found to have the same DNA sequence as gnl I dbest I 24254 T05200.

The PCR product generated using the hdx-1 and hdx-4 primers was then labeled and used to screen another human fetal brain cDNA library. The isolate was sequenced (SEQ ID NO:11) and the predicted protein determined (SEQ ID NO:12) (Figure 2A-C). Not greater than 107 continuous nucleotides of SEQ ID NO:11 were present in T05200. Applying standard techniques, the cDNA isolate obtained using the PCR product as probe was then labeled and itself used as a probe to screen a northern blot containing poly(A)$^+$ mRNA isolated from various human tissue samples. This probe was observed to hybridize to a 5.4-kb RNA in

heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. When this probe was used to screen a zoo blot (a blot containing genomic EcoRI-digested DNA of various species, obtained from Clontech) by Southern hybridization, hybridization was observed in genomic human, monkey, rat, mice, dog, cow, and yeast DNA. Hybridization was not observed in the rabbit and chicken genomic DNA.

Structural analysis of the human Deltex protein:

The predicted human Deltex product has 720 amino acids and an estimated molecular mass of approximately 80 kDa. The 180 amino terminal residues of human Deltex have an approximate identity of 33% with the corresponding amino acid residues of *Drosophila* Deltex and the nucleic acids encoding these amino acids have an approximate 52% identity. The 180 carboxy terminal amino acids of human Deltex have an approximate 48% identity with the corresponding amino acid residues of *Drosophila* Deltex and the nucleic acids encoding these carboxy terminal amino acids have an approximate 49% identity.

A structural analysis of human Deltex protein revealed a conserved structure among Deltex proteins (see Figure 3). Like *Drosophila* Deltex, human Deltex has both ring-H2-zinc finger (amino acids 411-471) (SEQ ID NO:25) and putative SH3-binding domains. Noticeably absent from the human Deltex are the two opa repeats that subdivide the primary structure of the *Drosophila* Deltex into three domains. Each of the *Drosophila* Deltex domains I, II, and III, has been found using the yeast "interaction trap assay" to be capable of mediating homotypic interactions (*see infra*).

i) Domain I:

Domain I corresponds to the N-terminal 303 amino acids of *Drosophila* and the first 237 amino acids of human Deltex. In *Drosophila*, we have demonstrated that the region of human Deltex corresponding to the first 175 amino acids of domain I is essential and sufficient to bind the Notch ANK repeats and overexpression of this domain can rescue loss-of-function phenotypes of Deltex. Since *Drosophila* Deltex can bind to human Notch-1 and 2, conservation

of binding activity between human Deltex and *Drosophila* domain I is suggested. Furthermore, this has been demonstrated. *See infra*.

ii) Putative SH3 binding domains:

Domain II of *Drosophila* Deltex contains a putative SH3-binding site (amino acids 476-484) (Matsuno et al., 1995, Development 121(8):2633-2644). Five putative SH3-binding sites (SEQ ID NOS:17-21) are found in human Deltex (within amino acids 226-377) in a position corresponding to the SH3-binding site in domain II of *Drosophila* Deltex (Table III).

SH2 and SH3 domains are conserved protein modules so named based on their homology to the oncogene Src (Src Homology). These motifs have been implicated in mediating protein-protein interactions in a number of signal transduction pathways (reviewed in Cell 71:359-362;Science 252:668-674; Trends Cell Biol. 3:8-13; FEBS 307:55-61). Recently, a complementary motif that binds to the SH3 domain has been identified and called simply an 'SH3-binding domain' ("SH3-BD") (Science 259:1157-1161). The core binding region of SH3-BD is proline-rich and approximately ten residues in length. As shown in Table III, this motif, as defined from a mouse protein that experimentally bound an SH3 domain (SEQ ID NO:23), is shown aligned to the putative SH3-binding site in *Drosophila* Deltex (SEQ ID NO:22) and the five regions (SEQ ID NOS: 17-21) that may represent human versions of this motif. These regions are located centrally in the Deltex protein. For reference, regions of the protein encoded by the *Drosophila Son of sevenless* (SOS) protein (SEQ ID NO:24), which may also contain SH3-BD, is shown. The Son of sevenless encoded protein, a putative guanine nucleotide exchange factor (GNEF), has been shown to bind to an 'adaptor' protein (drk) containing only SH2 and SH3 modules, although the actual residues that mediate binding have not been accurately defined (Simon M., et al., 1993, Cell 73:169-177 and Olivier J., et al., 1993, Cell 73:179-191).

TABLE III

Putative SH-3 Domain Binding Sites in Deltex Pr teins			SEQ ID NO:
Fly Deltex		RAP-VPPPLPLHPRQQ	22
Mouse 3BP-1		RAPTMPPPLPPVPPQP	23
Fly Son of sevenless		RA--VPPPLPPRRKER	24
Human Deltex			
I	226-244	VXPAPPLSXPXXPGGPPGA	17
II	271-285	XSPGXPPRSPGAPGG	18
III	311-322	SIPPGVPALPVK	19
IV	353-365	RAPKPILHPPPVS	20
V	368-377	VKPVPGVPGV	21

There are currently only six SH3-containing proteins identified in *Drosophila*, any one of which may be a direct binding partner of Deltex, and thus an indirect partner of Notch.

The functional requirement of the Deltex domain II-III in *Drosophila* which contains the putative SH3 domain binding site and ring-H2-zinc finger has been suggested from deletion analyses, in which the deletion of these domains resulted in a significant reduction of the ability to activate the Notch signaling pathway. These results indicate that domains II and III are not redundant.

iii) Ring-H2-zinc finger:

Human *deltex* (nucleotides 1734-1916 of SEQ ID NO: 11) encodes a ring-H2-zinc finger (SEQ ID NO: 25), appearing as amino acids 411-471 of SEQ ID NO:12 in that part of human Deltex which corresponds to domain III of *Drosophila* Deltex. This type of zinc finger is believed to be involved in protein/protein interactions.

6.2. MATERIAL AND METHODS

6.2.1. SEQUENCE DETERMINATION AND ANALYSIS

The *EcoR*I-cDNA insert was subcloned directly in both orientations into Bluescript KS. Overlapping deletions were produced on the insert using the DNAse I method to generate bidirectional deletions (Eberle et al., 1993, Biotechniques 14:408). The resulting deletions were analyzed using an IBI automatic sequencer.

DNA sequence manipulations were performed using Intelligenetic's PC-GENE software. Open reading frame prediction and plotting were performed using the University of Wisconsin program CODONPREFERENCE (Gribshov et al., 1984, Nucl. Acids Res. 12:539-549). The GenPept and SWISS-PROT databases were searched with all or part of the deduced amino acid sequence using the FASTA program (Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA, 85:2444-2448) available by the GenBank FASTA server through BITNET.

7. EXAMPLE: HUMAN DELTEX BINDS HUMAN AND DROSOPHILA NOTCH

We have demonstrated in *Drosophila* a specific and direct physical interaction between Deltex and Notch ANK repeats (Diederich et al., 1994, Development 120:473-481). To study protein-protein interactions between human Deltex and various cytoplasmic domains of human and *Drosophila* Notch receptors, we conducted expression studies in yeast using the so-called 'interaction trap' assay technique (Zervos et al., 1993, Cell 72:223-232).

In this assay, one protein segment is fused to the DNA-binding domain of the LexA protein, which in turn binds to the promoter of a *LexAop-lacZ* reporter construct without activating transcription. These constructs are referred as pEG. A second foreign protein segment is fused to an acidic transcriptional activation domain that does not bind DNA on its own. These constructs are referred to as pJG. Coexpression of these two proteins in yeast cells results in the functional reconstruction of an active LexA "hybrid" transcription factor if the foreign proteins physically interact with one another. Activity of the hybrid transcription factor is monitored by transcription of the β-galactosidase reporter gene. Expression of fusion proteins from the pJG construct is induced when yeast cells

are cultured in the galactose media but not in the glucose media. Therefore, positive interaction should be observed only in galactose media.

The constructs examined using the yeast interaction were as follows: the pEGhDeltex construct contains the entire coding region of human Deltex; pJGhNotch-1 encodes the ankyrin repeats region of human Notch-1 from amino acids 1826-2147; pJGhNotch-2 encodes the ankyrin repeats region of human Notch-2 from amino acids 1772-2084; pJGhNotch encodes the ankyrin repeats of *Drosophila* Notch from amino acids 1827-2259; and JGfHairless contains the entire coding region of *Drosophila* Hairless.

As presented in Table IV, significant induction of β-galactosidase activity was observed when yeast cells cotransfected with pEGhDeltex and pJGhNotch-1, pJGHNotch-2 or pJGHfNotch, were cultured in galactose media (Table IV). These results indicate that human Deltex binds to the ankyrin repeats human Notch-1, human Notch-2 as well as that of *Drosophila* Notch. Standard deviation is presented in the parentheses.

TABLE IV

	Media	
Coexpressed Constructs	Galactose	Glucose
pEGhDeltex/pJGhNotch-1	732(35)	4(1)
pEGhDeltex/pJGhNotch-2	195(25)	11(5)
pEGhDeltex/pJGfNotch	892(184)	14(4)
pEGhDeltex/pJGHairless	61(13)	20(2)
pEGhDeltex/pJG	38(1)	21(5)
pEG/pJGhNotch-1	47(13)	13(5)
pEG/pJGhNotch-2	48(7)	19(8)
pEG/pJGfNotch	69(18)	20(5)
pEG/pJGHairless	59(4)	39(14)
pEG/pJG	60(10)	32(4)

8. EXAMPLE: CLONING OF VERTEBRATE DELTEX GENES

The evolution of humans and *Drosophila* diverged about 600 million years ago. As discussed *supra*, Deltex protein demonstrates a conserved structure in these two evolutionary distant species. Knowledge of the conserved regions of the protein allows one to design synthetic degenerate primers for use in hybridization and PCR reactions which enable the cloning of Deltex encoding nucleic acids in other organisms.

Five regions of high conservation between human and *Drosophila* are found in amino acid stretches of human Deltex amino acid numbers 414-419 (SEQ ID NO:30), 475-480 (SEQ ID NO:31), 504-511 (SEQ ID NO:32), 531-539 (SEQ ID NO:33) and 557-564 (SEQ ID NO:34). These sequences are conserved in *Drosophila* Deltex amino acid stretches 549-555 (SEQ ID NO:35), 603-608 (SEQ ID NO:36), 632-639 (SEQ ID NO:37), 659-667 (SEQ ID NO:38) and 685-692 (SEQ ID NO:39), respectively. Conserved amino acid stretches may be used alone or in combination to isolate the *deltex* encoding nucleic acids of other organisms.

By way of example, a murine *deltex* gene is obtained as follows: Standard techniques are utilized to synthesize a series of degenerate primers encoding amino acids 414-419 in *Drosophila* (SEQ ID NO:30) and 549-555 in human (SEQ ID NO:35) in a 5' to 3' orientation. A second series of degenerate primers corresponding to the antisense strand of the nucleic acids encoding amino acids 475-480 in *Drosophila* (SEQ ID NO:31) and 603-608 in human (SEQ ID NO:36) is also synthesized. The two series of primers are added to a mixture containing mouse embryonic cDNA as template for the PCR amplification. PCR is carried out at a range of stringencies, according to methods commonly known, to allow for varying degrees of nucleotide similarity between the known *deltex* sequences and the mouse nucleic acid homolog being isolated.

After successful PCR amplification, the segment of mouse *deltex* gene is molecularly cloned and sequenced through techniques known in the art. This segment is used as a probe to isolate a complete cDNA and genomic clone. The complete nucleotide sequence of the mouse *deltex* homolog is determined by sequence analysis.

9. DEPOSIT OF MICROORGANISMS

Plasmid pBS hdx containing a cDNA insert encoding a full-length human *deltex* as a EcoRI insert in Bluescript vector (Stratagene) was deposited by S. Leslie Misrock, of Pennie & Edmonds, 1155 Avenue of the Americas, New York, New York 10036 on behalf of Yale University on November 17, 1995, with the American Type Culture Collection, 1201 Parklawn Drive, Rockville, Maryland 20852, under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures, and assigned accession number 97341.

The present invention is not to be limited in scope by the microorganism deposited or the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated in their entireties.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Artavanis-Tsakonas, Spyridon
Matsuno, Kenji

(ii) TITLE OF INVENTION: VERTEBRATE DELTEX PROTEINS, NUCLEIC ACIDS, AND ANTIBODIES, AND RELATED METHODS AND COMPOSITIONS

(iii) NUMBER OF SEQUENCES: 39

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: U.S.A.
(F) ZIP: 10036-2711

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: To Be Assigned
(B) FILING DATE: 22-NOV-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Misrock, S. Leslie
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3771 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 345..2555

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AAATGCTAGA AAAACCGTTT TTACCATCAA ACGTGAATTC TTAAGCTGCG CCTAAACGAA 60

ACCGAGTGAC TAAAGAACCA GAACGAAAAC TTCGGGAAAA TGGAAGCCAG GGAAAATCAG 120

GGATAACTAA CGCTGGCAGC GGGTCCACCA TTTTTAATTT CTTTGTTTAT TTTGTGCCCA 180

TCTTCGCGAG CGAGCGAGAT AGCGCGACAG CAACAGCAAG AGAGAGCGAG AGAGAGAGTG 240

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AGTGAGTGAG AGCTAGTGAA GAGAGCGCAG GAGGAGTTGG ATATGGAAAT GGGCATGGAT      300

ATGGCAATGG GCTCACTCCA CGGATAACGG ATCAACTGCA AGCA ATG GCC AGC AGC       356
                                                Met Ala Ser Ser
                                                  1

GCC GGA AGT GCG GCA TCC GGA TCC GTT GTT CCC GGT GGC GGA GGT AGC        404
Ala Gly Ser Ala Ala Ser Gly Ser Val Val Pro Gly Gly Gly Gly Ser
  5                  10                  15                  20

GCC GCC TCC AGT TGT GCC ACC ATG GCC CTG TCC ACC GCC GGA TCC GGT        452
Ala Ala Ser Ser Cys Ala Thr Met Ala Leu Ser Thr Ala Gly Ser Gly
                 25                  30                  35

GGG CCG CCC GTG AAC CAC GCC CAC GCC GTC TGC GTG TGG GAG TTC GAG        500
Gly Pro Pro Val Asn His Ala His Ala Val Cys Val Trp Glu Phe Glu
             40                  45                  50

TCG CGC GGC AAG TGG CTG CCC TAT TCG CCG GCG GTG TCG CAG CAC TTG        548
Ser Arg Gly Lys Trp Leu Pro Tyr Ser Pro Ala Val Ser Gln His Leu
         55                  60                  65

GAA CGC GCC CAC GCC AAG AAA CTG ACG CGC GTC ATG CTG AGC GAT GCG        596
Glu Arg Ala His Ala Lys Lys Leu Thr Arg Val Met Leu Ser Asp Ala
     70                  75                  80

GAT CCC AGC CTG GAG CAG TAC TAC GTC AAC GTG CGC ACA ATG ACC CAG        644
Asp Pro Ser Leu Glu Gln Tyr Tyr Val Asn Val Arg Thr Met Thr Gln
 85                  90                  95                  100

GAA TCG GAG GCG GAA ACG CGC TCC GGC CTG CTG ACC ATC GGT GTT CGG        692
Glu Ser Glu Ala Glu Thr Arg Ser Gly Leu Leu Thr Ile Gly Val Arg
                105                 110                 115

CGC ATG TTA TAC GCA CCC AGC TCG CCG GCG GGC AAG GGC ACC AAG TGG        740
Arg Met Leu Tyr Ala Pro Ser Ser Pro Ala Gly Lys Gly Thr Lys Trp
            120                 125                 130

GAG TGG TCG GGC GGC AGT GCC GAT AGC AAC AAC GAC TGG CGG CCC TAC        788
Glu Trp Ser Gly Gly Ser Ala Asp Ser Asn Asn Asp Trp Arg Pro Tyr
        135                 140                 145

AAC ATG CAC GTC CAG TGC ATC ATC GAG GAC GCC TGG GCG AGG GGC GAA        836
Asn Met His Val Gln Cys Ile Ile Glu Asp Ala Trp Ala Arg Gly Glu
    150                 155                 160

CAA ACC TTG GAC CTG TGC AAC ACC CAC ATC GGC CTG CCG TAC ACC ATT        884
Gln Thr Leu Asp Leu Cys Asn Thr His Ile Gly Leu Pro Tyr Thr Ile
165                 170                 175                 180

AAT TTT TGC AAT CTC ACC CAC GTG CGC CAA CCC AGC GGA CCC ATG CGC        932
Asn Phe Cys Asn Leu Thr His Val Arg Gln Pro Ser Gly Pro Met Arg
                185                 190                 195

AGC ATT CGG CGT ACC CAA CAG GCG CCG TAT CCC TTG GTG AAA CTA ACG        980
Ser Ile Arg Arg Thr Gln Gln Ala Pro Tyr Pro Leu Val Lys Leu Thr
            200                 205                 210

CCA CAA CAG GCC AAC CAA CTC AAG TCG AAT TCC GCC AGC GTG AGC AGC       1028
Pro Gln Gln Ala Asn Gln Leu Lys Ser Asn Ser Ala Ser Val Ser Ser
        215                 220                 225

CAG TAC AAC ACT CTA CCC AAA CTG GGC GAC ACC AAG AGC CTG CAC AGA       1076
Gln Tyr Asn Thr Leu Pro Lys Leu Gly Asp Thr Lys Ser Leu His Arg
    230                 235                 240

GTG CCC ATG ACC AGG CAA CAG-CAC CCA TTG CCC ACC AGC CAT CAA GTG       1124
Val Pro Met Thr Arg Gln Gln His Pro Leu Pro Thr Ser His Gln Val
245                 250                 255                 260
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CAG CAG CAG CAG CAT CAG CTC CAG CAT CAA CAG CAG CAG CAG CAG CAA      1172
Gln Gln Gln Gln His Gln Leu Gln His Gln Gln Gln Gln Gln Gln Gln
                265                 270                 275

CAT CAT CAC CAG CAT CAG CAA CAA CAG CAT CAG CAA CAG CAG CAA CAT      1220
His His His Gln His Gln Gln Gln Gln His Gln Gln Gln Gln Gln His
            280                 285                 290

CAG ATG CAG CAC CAT CAG ATC CAT CAT CAG ACG GCG CCC AGG AAG CCG      1268
Gln Met Gln His His Gln Ile His His Gln Thr Ala Pro Arg Lys Pro
        295                 300                 305

CCC AAG AAG CAC AGC GAG ATC TCC ACC ACC AAT CTA CGC CAG ATA CTC      1316
Pro Lys Lys His Ser Glu Ile Ser Thr Thr Asn Leu Arg Gln Ile Leu
    310                 315                 320

AAC AAC CTA AAC ATC TTC AGC AGC AGC ACT AAG CAC CAA TCG AAC ATG      1364
Asn Asn Leu Asn Ile Phe Ser Ser Ser Thr Lys His Gln Ser Asn Met
325                 330                 335                 340

TCG ACG GCG GCC AGT GCC AGT TCA TCC TCC TCA TCG GCC TCG CTG CAC      1412
Ser Thr Ala Ala Ser Ala Ser Ser Ser Ser Ser Ser Ala Ser Leu His
                345                 350                 355

CAT GCC AAC CAT CTG TCG CAT GCG CAC TTT TCG CAC GCC AAG AAC ATG      1460
His Ala Asn His Leu Ser His Ala His Phe Ser His Ala Lys Asn Met
            360                 365                 370

CTG ACT GCC TCG ATG AAC AGT CAT CAT AGT CGC TGC TCG GAG GGA TCG      1508
Leu Thr Ala Ser Met Asn Ser His His Ser Arg Cys Ser Glu Gly Ser
        375                 380                 385

CTG CAG TCG CAA AGG AGC AGC CGG ATG GGC TCG CAT CGC TCG AGA TCG      1556
Leu Gln Ser Gln Arg Ser Ser Arg Met Gly Ser His Arg Ser Arg Ser
    390                 395                 400

CGA ACG CGG ACC TCG GAC ACG GAC ACG AAC AGT GTG AAA TCG CAT CGG      1604
Arg Thr Arg Thr Ser Asp Thr Asp Thr Asn Ser Val Lys Ser His Arg
405                 410                 415                 420

CGG AGA CCC AGT GTG GAC ACC GTG TCC ACT TAC CTC AGC CAC GAG AGC      1652
Arg Arg Pro Ser Val Asp Thr Val Ser Thr Tyr Leu Ser His Glu Ser
                425                 430                 435

AAG GAG AGC CTG CGC AGC AGG AAC TTT GCC ATT TCG GTC AAT GAT CTG      1700
Lys Glu Ser Leu Arg Ser Arg Asn Phe Ala Ile Ser Val Asn Asp Leu
            440                 445                 450

CTG GAC TGC TCG CTT GGC AGC GAT GAA GTT TTT GTG CCC TCC GTG CCG      1748
Leu Asp Cys Ser Leu Gly Ser Asp Glu Val Phe Val Pro Ser Val Pro
        455                 460                 465

CCA TCG TCG CTG GGC GAA AGG GCG CCG GTG CCG CCG CCA TTA CCA CTG      1796
Pro Ser Ser Leu Gly Glu Arg Ala Pro Val Pro Pro Pro Leu Pro Leu
    470                 475                 480

CAT CCG CGA CAG CAA CAG CAG CAG CAA CAA CAG CAG CAA CAG CTG CAG      1844
His Pro Arg Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Leu Gln
485                 490                 495                 500

ATG CAA CAG CAG CAA CAG GCG CAG CAG CAG CAG CAG CAA TCA ATC GCC      1892
Met Gln Gln Gln Gln Gln Ala Gln Gln Gln Gln Gln Gln Ser Ile Ala
                505                 510                 515

GGT TCG ATT GTG GGC GTG GAC CCG GCC AGC GAT ATG ATA TCG CGT TTT      1940
Gly Ser Ile Val Gly Val Asp Pro Ala Ser Asp Met Ile Ser Arg Phe
            520                 525                 530

GTC AAG GTG GTG GAG CCA CCG CTG TGG CCC AAT GCC CAG CCC TGT CCC      1988
Val Lys Val Val Glu Pro Pro Leu Trp Pro Asn Ala Gln Pro Cys Pro
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        535                 540                 545

ATG TGC ATG GAG GAG CTG GTG CAC TCC GCC CAG AAT CCG GCC ATT TCG      2036
Met Cys Met Glu Glu Leu Val His Ser Ala Gln Asn Pro Ala Ile Ser
    550                 555                 560

CTG AGT CGC TGC CAG CAT CTC ATG CAT TTG CAG TGC CTC AAT GGG ATG      2084
Leu Ser Arg Cys Gln His Leu Met His Leu Gln Cys Leu Asn Gly Met
565                 570                 575                 580

ATA ATT GCC CAG CAA AAC GAA ATG AAC AAG AAC CTT TTC ATC GAG TGC      2132
Ile Ile Ala Gln Gln Asn Glu Met Asn Lys Asn Leu Phe Ile Glu Cys
                585                 590                 595

CCT GTA TGC GGC ATC GTT TAC GGC GAG AAG GTC GGC AAT CAG CCC ATT      2180
Pro Val Cys Gly Ile Val Tyr Gly Glu Lys Val Gly Asn Gln Pro Ile
            600                 605                 610

GGC AGC ATG TCG TGG AGC ATA ATT AGC AAG AAT CTG CCA GGA CAC GAG      2228
Gly Ser Met Ser Trp Ser Ile Ile Ser Lys Asn Leu Pro Gly His Glu
        615                 620                 625

GGT CAG AAC ACC ATA CAG ATT GTT TAC GAC ATT GCA TCG GGA CTG CAG      2276
Gly Gln Asn Thr Ile Gln Ile Val Tyr Asp Ile Ala Ser Gly Leu Gln
    630                 635                 640

ACG GAG GAG CAT CCG CAT CCA GGT CGT GCC TTC TTC GCC GTG GGA TTC      2324
Thr Glu Glu His Pro His Pro Gly Arg Ala Phe Phe Ala Val Gly Phe
645                 650                 655                 660

CCG CGG ATC TGC TAC TTG CCG GAC TGC CCG CTG GGG CGA AAG GTT TTG      2372
Pro Arg Ile Cys Tyr Leu Pro Asp Cys Pro Leu Gly Arg Lys Val Leu
                665                 670                 675

CGC TTC CTC AAG ATT GCA TTC GAT CGT CGG CTG CTT TTC TCG ATC GGA      2420
Arg Phe Leu Lys Ile Ala Phe Asp Arg Arg Leu Leu Phe Ser Ile Gly
            680                 685                 690

CGA TCG GTG ACC ACC GGA CGC GAG GAT GTG GTG ATC TGG AAC AGT GTG      2468
Arg Ser Val Thr Thr Gly Arg Glu Asp Val Val Ile Trp Asn Ser Val
        695                 700                 705

GAT CAC AAG ACG CAG TTC AAT ATG TTT CCG GAT CCC ACC TAT TTG CAG      2516
Asp His Lys Thr Gln Phe Asn Met Phe Pro Asp Pro Thr Tyr Leu Gln
    710                 715                 720

CGA ACC ATG CAA CAG CTG GTG CAC CTG GGC GTG ACG GAT TAAGGATTAG      2565
Arg Thr Met Gln Gln Leu Val His Leu Gly Val Thr Asp
725                 730                 735

TTCCCTGTCC CCAAGTAGAA CTACCAACCA ACCAATCAAC CACCCACCCA CCGAAGTCCC    2625

CTCGATCATT CTCTTCCATT CGTCGTTAAG TTACTTTCTA CATAATCTCA GTGTGTGTGC    2685

AATCCTCGTT TACTATGATA TATTTTTTTT ATAGATATAT TGTAATAGCG TTCGAGCTGC    2745

TCGAACCCTA AAACAACAGC AAACCACAAT TGCAATTGTA GCTTCCTTTC CGCTCTTCCA    2805

ATTCGTATTT GTACGCACAT ACGCAATAAG TTGGCGTACA TCATATGTAT TAGCTAGTTA    2865

GTTAGTTAGT TAGTTAGTTG TAGCTGTAGT TCCCAAGAGA ATCTTGACCC AAGACACCTA    2925

CTAGTATTAG GCATTATCCT GATTCTTGAT TCCTGATTCG ATTCAAGCCA AGCCAAGCCA    2985

CGCCATTCGA GTGCAAGCTG TGCCAAAATC GTAGCGCTCC CGTTTATAGG ATATGTATAT    3045

TGTTGATATA GCTAGCTATA ACCATTGCCC ATCTCTCCAT CTCTCTCGGT TTCGAATTTG    3105

TCTCTTTCAT CAGATCCATG TGAATTTTCT TTATATCGGA TTTATATAGG ATTAAAATAG    3165
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TATTTTGAGA GAGGAAATGG AGATGGGTAA ATTCGATAGA CTTGTCTCAC TTGTCTTGGC    3225
CATTTAATCT CTTTCATTCA GCGAATTTGA TGTGATTTTA ATTTGAATTA TTCATTATTA    3285
AACGGAGCAT TTAGGAAGCA TAGTTGTAAC GCAGCCAGAT ATTCCATTAC GCATATACAT    3345
ATACATATAC ATATACATAC ATACATAAAC ATATTTTAAC ATAGCCCCAT AGCCATACGA    3405
CATAACAATA ATTTTTTTTA TCGAATCCCT TGCATACATT TGATGAATTG TTGCTTTCAT    3465
ATTGATATCA TCGAGCATCG AACGAACTAT CGTATACATC GCCAATATAT AGCATATATA    3525
GCATATAGTA TGTAGAGATC GTACGGACAG CTAGCGGCTA CTGACCGCGC CACCATATTT    3585
GATATGATAT GATATGATTT TACTAAGTTG TATTTAGCAC TGATTAGTTA TTAAAGTTCA    3645
TTTGACGAAT ATTCCACAAC AAATTCCACA CCATTTATGT ATGCATATTA CGCATATATA    3705
ATACAGTACA TTTATATATA GTTCAAATAA AGTAACTTCA TTCATGTTCA AAAAAAAAAA    3765
AAAAAA                                                               3771
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 737 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ala Ser Ser Ala Gly Ser Ala Ala Ser Gly Ser Val Val Pro Gly
 1               5                  10                  15

Gly Gly Gly Ser Ala Ala Ser Ser Cys Ala Thr Met Ala Leu Ser Thr
            20                  25                  30

Ala Gly Ser Gly Gly Pro Pro Val Asn His Ala His Ala Val Cys Val
        35                  40                  45

Trp Glu Phe Glu Ser Arg Gly Lys Trp Leu Pro Tyr Ser Pro Ala Val
    50                  55                  60

Ser Gln His Leu Glu Arg Ala His Ala Lys Lys Leu Thr Arg Val Met
65                  70                  75                  80

Leu Ser Asp Ala Asp Pro Ser Leu Glu Gln Tyr Tyr Val Asn Val Arg
                85                  90                  95

Thr Met Thr Gln Glu Ser Glu Ala Glu Thr Arg Ser Gly Leu Leu Thr
            100                 105                 110

Ile Gly Val Arg Arg Met Leu Tyr Ala Pro Ser Ser Pro Ala Gly Lys
        115                 120                 125

Gly Thr Lys Trp Glu Trp Ser Gly Gly Ser Ala Asp Ser Asn Asn Asp
    130                 135                 140

Trp Arg Pro Tyr Asn Met His Val Gln Cys Ile Ile Glu Asp Ala Trp
145                 150                 155                 160

Ala Arg Gly Glu Gln Thr Leu Asp Leu Cys Asn Thr His Ile Gly Leu
                165                 170                 175

Pro Tyr Thr Ile Asn Phe Cys Asn Leu Thr His Val Arg Gln Pro Ser
            180                 185                 190
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Gly Pro Met Arg Ser Ile Arg Arg Thr Gln Gln Ala Pro Tyr Pro Leu
195 200 205

Val Lys Leu Thr Pro Gln Gln Ala Asn Gln Leu Lys Ser Asn Ser Ala
210 215 220

Ser Val Ser Ser Gln Tyr Asn Thr Leu Pro Lys Leu Gly Asp Thr Lys
225 230 235 240

Ser Leu His Arg Val Pro Met Thr Arg Gln Gln His Pro Leu Pro Thr
245 250 255

Ser His Gln Val Gln Gln Gln Gln His Gln Leu Gln His Gln Gln Gln
260 265 270

Gln Gln Gln Gln His His His Gln His Gln Gln Gln Gln His Gln Gln
275 280 285

Gln Gln Gln His Gln Met Gln His His Gln Ile His His Gln Thr Ala
290 295 300

Pro Arg Lys Pro Pro Lys Lys His Ser Glu Ile Ser Thr Thr Asn Leu
305 310 315 320

Arg Gln Ile Leu Asn Asn Leu Asn Ile Phe Ser Ser Ser Thr Lys His
325 330 335

Gln Ser Asn Met Ser Thr Ala Ala Ser Ala Ser Ser Ser Ser Ser Ser
340 345 350

Ala Ser Leu His His Ala Asn His Leu Ser His Ala His Phe Ser His
355 360 365

Ala Lys Asn Met Leu Thr Ala Ser Met Asn Ser His His Ser Arg Cys
370 375 380

Ser Glu Gly Ser Leu Gln Ser Gln Arg Ser Ser Arg Met Gly Ser His
385 390 395 400

Arg Ser Arg Ser Arg Thr Arg Thr Ser Asp Thr Asp Thr Asn Ser Val
405 410 415

Lys Ser His Arg Arg Arg Pro Ser Val Asp Thr Val Ser Thr Tyr Leu
420 425 430

Ser His Glu Ser Lys Glu Ser Leu Arg Ser Arg Asn Phe Ala Ile Ser
435 440 445

Val Asn Asp Leu Leu Asp Cys Ser Leu Gly Ser Asp Glu Val Phe Val
450 455 460

Pro Ser Val Pro Pro Ser Ser Leu Gly Glu Arg Ala Pro Val Pro Pro
465 470 475 480

Pro Leu Pro Leu His Pro Arg Gln Gln Gln Gln Gln Gln Gln Gln Gln
485 490 495

Gln Gln Leu Gln Met Gln Gln Gln Gln Gln Ala Gln Gln Gln Gln Gln
500 505 510

Gln Ser Ile Ala Gly Ser Ile Val Gly Val Asp Pro Ala Ser Asp Met
515 520 525

Ile Ser Arg Phe Val Lys Val Val Glu Pro Pro Leu Trp Pro Asn Ala
530 535 540

Gln Pro Cys Pro Met Cys Met Glu Glu Leu Val His Ser Ala Gln Asn
545 550 555 560

Pro Ala Ile Ser Leu Ser Arg Cys Gln His Leu Met His Leu Gln Cys

565 570 575

Leu Asn Gly Met Ile Ile Ala Gln Gln Asn Glu Met Asn Lys Asn Leu
580 585 590

Phe Ile Glu Cys Pro Val Cys Gly Ile Val Tyr Gly Glu Lys Val Gly
595 600 605

Asn Gln Pro Ile Gly Ser Met Ser Trp Ser Ile Ile Ser Lys Asn Leu
610 615 620

Pro Gly His Glu Gly Gln Asn Thr Ile Gln Ile Val Tyr Asp Ile Ala
625 630 635 640

Ser Gly Leu Gln Thr Glu Glu His Pro His Pro Gly Arg Ala Phe Phe
645 650 655

Ala Val Gly Phe Pro Arg Ile Cys Tyr Leu Pro Asp Cys Pro Leu Gly
660 665 670

Arg Lys Val Leu Arg Phe Leu Lys Ile Ala Phe Asp Arg Arg Leu Leu
675 680 685

Phe Ser Ile Gly Arg Ser Val Thr Thr Gly Arg Glu Asp Val Val Ile
690 695 700

Trp Asn Ser Val Asp His Lys Thr Gln Phe Asn Met Phe Pro Asp Pro
705 710 715 720

Thr Tyr Leu Gln Arg Thr Met Gln Gln Leu Val His Leu Gly Val Thr
725 730 735

Asp

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gln Pro Cys Pro Met Cys Met Glu Glu Leu Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Glu Asp Cys Thr Ile Cys Met Glu Arg Leu Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Leu Ser Arg Cys Gln His Leu Met His Leu Gln Cys Leu Asn Gly Met
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Gly Arg Cys Gly His Met Tyr His Leu Leu Cys Leu Val Ala Met
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ile Ile Ala Gln Gln Asn Glu Met Asn Lys Asn Leu Phe Ile Glu Cys
1 5 10 15

Pro Val Cys Gly Ile Val Tyr Gly Glu Lys Val Gly Asn Gln Pro Ile
20 25 30

Gly Ser Met Ser
35

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Leu Val Ala Met Tyr Ser Asn Gly Asn Lys Asp Gly Ser Leu Gln Cys
1 5 10 15

Pro Thr Cys Lys Pro Ser Met Gly Arg Arg Arg Val Arg Ser Arg Leu
20 25 30

Gly Arg Trp Ser
35

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Val Tyr Gly Glu Lys Val Gly Val Gln Pro Ile Gly Ser Met Ser Trp
1 5 10 15

Ser Ile Ile Ser Lys Asn Leu Pro Gly His Glu Gly Gln Asn Thr Ile
20 25 30

Gln Ile Val Tyr Asp
35

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ile Tyr Gly Glu Lys Thr Gly Thr Gln Pro Pro Gly Lys Met Glu Phe
1 5 10 15

His Leu Ile Pro His Ser Leu Xaa Phe Gly Pro Asp Thr Gln Thr Xaa
20 25 30

Arg Ile Val Tyr Asp
35

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2547 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 504..2363

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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GCGAGAAGCC CCACTGAAGC CGGGCGCAGG GTCTGGGACG CAGTTGGGAG TGCAAAGGGC      60

TGGCTGAGAG CCGCAGGAGC AGCAGGCTGT GGCCCAGGCC TCCTGGGTGA CAGGCCCTGT     120

CTGGCGGGGA ACAGGGACCA AGAGACAACA CAGAAGAGGC TGGACCTCGA ACAGGGGCGG     180

CTGCCTCACT CCCTACCTGA GCCAGCCGAG GGGGCCAAGG ACTTTAGAGC TGTTTCCTCC     240

GGCATAAGAG AGACACTTGC TTTCCAGGGC AGCACCCTTT ATCGGAGAAG GCTCTACAGG     300

GAAGGGGTCT TTGCAGCCTG GATGGCCATC CCACATTCCT TTAACGGAGG TCTCTAGGCC     360

TCAGAGAGAA CCCAGAGTTA GAAAGGAGGC CAGACGGTCC TTGCTGTCCC CCTGGGGAGA     420

GAGGAAGTTG CCGCCTGCTG CCAGGCCCAG GAGGAGCTGG GCCTGCAATA GTGGGGGACC     480

TGGCCCCTGA GGCAGTGGCG GCC ATG TCA CGG CCA GGC CAC GGT GGG CTG           530
                         Met Ser Arg Pro Gly His Gly Gly Leu
                           1               5

ATG CCT GTG AAT GGT CTG GGC TTC CCA CCG CAG AAC GTG GCC CGG GTG        578
Met Pro Val Asn Gly Leu Gly Phe Pro Pro Gln Asn Val Ala Arg Val
 10                  15                  20                  25

GTG GTG TGG GAG TGT CTG AAT GAG CAC AGC CGC TGG CGG CCC TAC ACG        626
Val Val Trp Glu Cys Leu Asn Glu His Ser Arg Trp Arg Pro Tyr Thr
             30                  35                  40

GCC ACC GTG TGC CAC CAC ATT GAG AAC GTG CTG AAG GAG GAC GCT CGC        674
Ala Thr Val Cys His His Ile Glu Asn Val Leu Lys Glu Asp Ala Arg
         45                  50                  55

GGT TCC GTG GTC CTG GGG CAG GTG GAC GCC CAG CTT GTG CCC TAC ATC        722
Gly Ser Val Val Leu Gly Gln Val Asp Ala Gln Leu Val Pro Tyr Ile
     60                  65                  70

ATC GAC CTG CAG TCC ATG CAC CAG TTT CGC CAG GAC ACA GGC ACC ATG        770
Ile Asp Leu Gln Ser Met His Gln Phe Arg Gln Asp Thr Gly Thr Met
 75                  80                  85

CGG CCC GTG CGG CGC AAC TTC TAC GAC CCG TCG TCG GCG CCG GGC AAG        818
Arg Pro Val Arg Arg Asn Phe Tyr Asp Pro Ser Ser Ala Pro Gly Lys
 90                  95                 100                 105

GGC ATC GTG TGG GAG TGG GAG AAC GAC GGC GGC GCA TGG ACG GCC TAC        866
Gly Ile Val Trp Glu Trp Glu Asn Asp Gly Gly Ala Trp Thr Ala Tyr
            110                 115                 120

GAT ATG GAC ATC TGC ATC ACC ATC CAG AAC GCC TAC GAG AAG CAG CAC        914
Asp Met Asp Ile Cys Ile Thr Ile Gln Asn Ala Tyr Glu Lys Gln His
        125                 130                 135

CCG TGG CTC GAC CTC TCA TCG CTA GGC TTC TGC TAC CTC ATC TAC TTC        962
Pro Trp Leu Asp Leu Ser Ser Leu Gly Phe Cys Tyr Leu Ile Tyr Phe
    140                 145                 150

AAC AGC ATG TCG CAG ATG ARC CGC CAG ACG CGC CGG CGC CGC CGT CTG       1010
Asn Ser Met Ser Gln Met Xaa Arg Gln Thr Arg Arg Arg Arg Arg Leu
155                 160                 165

CGC CGC CGC CTG GAC CTC GCC TAC CCG CTC ACC GTG GGC TCC ATC CCT       1058
Arg Arg Arg Leu Asp Leu Ala Tyr Pro Leu Thr Val Gly Ser Ile Pro
170                 175                 180                 185

AAG TCG CAG TCG TGG CCC GTG GGT GBC AGC TCG GGH CAG CCC TGC TCC       1106
Lys Ser Gln Ser Trp Pro Val Gly Xaa Ser Ser Gly Gln Pro Cys Ser
            190                 195                 200
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TGM CAG CAG TGC CTG YTG GTC AAC AGC ACG CGC GCC GTC TCC AAC GTC      1154
Xaa Gln Gln Cys Leu Leu Val Asn Ser Thr Arg Ala Val Ser Asn Val
            205                 210                 215

ATC CTG GYC TCG CAG CGT CGT AAG GTG MCC CCC GCG CCC CCG CTG TCG      1202
Ile Leu Xaa Ser Gln Arg Arg Lys Val Xaa Pro Ala Pro Pro Leu Ser
        220                 225                 230

YCG CCG YCG MCA CCT GGA GGG CCT CCA GGC GCG CTT GGC GTG CGC CCC      1250
Xaa Pro Xaa Xaa Pro Gly Gly Pro Pro Gly Ala Leu Gly Val Arg Pro
    235                 240                 245

AGY GTC ACC TTC ACA GGC GNC GNG CTC TGN GAA GTG NNN TTC NAC GGT      1298
Ser Val Thr Phe Thr Gly Xaa Xaa Leu Xaa Glu Val Xaa Phe Xaa Gly
250                 255                 260                 265

CCC GTC GAG CCC GMG YCG TCT CCC GGG GYG CCC CCA CGG AGC CCG GGC      1346
Pro Val Glu Pro Xaa Xaa Ser Pro Gly Xaa Pro Pro Arg Ser Pro Gly
                270                 275                 280

GCC CCC GGC GGA GCG CGC ACC CCG GGG CAG AAC AAC CTC AAC CGG BCC      1394
Ala Pro Gly Gly Ala Arg Thr Pro Gly Gln Asn Asn Leu Asn Arg Xaa
            285                 290                 295

GGG CCC CAG CGC ACC ACC AGH GTG AGC GCG CGC GCC TCC ATC CCG CCG      1442
Gly Pro Gln Arg Thr Thr Xaa Val Ser Ala Arg Ala Ser Ile Pro Pro
        300                 305                 310

GGG GTC CCC GCA CTC CCG GTG AAG AAC TTG AAT GGT ACT GGG CCG GTC      1490
Gly Val Pro Ala Leu Pro Val Lys Asn Leu Asn Gly Thr Gly Pro Val
    315                 320                 325

CAT CCG GCC CTG GCA GGG ATG ACC GGG ATA CTG CTG TGC GCG GCC GGG      1538
His Pro Ala Leu Ala Gly Met Thr Gly Ile Leu Leu Cys Ala Ala Gly
330                 335                 340                 345

CTG CCC GTG TGC CTG ACG CGG GCC CCC AAG CCC ATC CTG CAC CCG CCG      1586
Leu Pro Val Cys Leu Thr Arg Ala Pro Lys Pro Ile Leu His Pro Pro
                350                 355                 360

CCC GTG AGC AAG AGC GAC GTG AAG CCC GTG CCT GGC GTG CCC GGG GTG      1634
Pro Val Ser Lys Ser Asp Val Lys Pro Val Pro Gly Val Pro Gly Val
            365                 370                 375

TGC CGC AAG ACC AAG AAG AAG CAC CTT AAA AAG AGT AAG AAT CCC GAG      1682
Cys Arg Lys Thr Lys Lys Lys His Leu Lys Lys Ser Lys Asn Pro Glu
        380                 385                 390

GAT GTG GTT CGA AGA TAC ATG CAG AAG GTG AAA AAC CCA CCT GAT GAG      1730
Asp Val Val Arg Arg Tyr Met Gln Lys Val Lys Asn Pro Pro Asp Glu
    395                 400                 405

GAC TGC ACC ATC TGC ATG GAG CGA CTG GTC ACA GCA TCA GGC TAC GAG      1778
Asp Cys Thr Ile Cys Met Glu Arg Leu Val Thr Ala Ser Gly Tyr Glu
410                 415                 420                 425

GGC GTG CTT CGG CAC AAG GGC GTG CGG CCT GAG CTC GTG GGC CGC CTG      1826
Gly Val Leu Arg His Lys Gly Val Arg Pro Glu Leu Val Gly Arg Leu
                430                 435                 440

GGC CGC TGT GGC CAC ATG TAC CAC CTG CTG TGC CTC GTG GCC ATG TAC      1874
Gly Arg Cys Gly His Met Tyr His Leu Leu Cys Leu Val Ala Met Tyr
            445                 450                 455

TCC AAT GGC AAC AAG GAT GGC AGC CTG CAG TGC CCC ACC TGC AAG GCC      1922
Ser Asn Gly Asn Lys Asp Gly Ser Leu Gln Cys Pro Thr Cys Lys Ala
        460                 465                 470

ATC TAC GGG GAG AAG ACG GGT ACG CAG CCG CCT GGG AAG ATG GAG TTC      1970
Ile Tyr Gly Glu Lys Thr Gly Thr Gln Pro Pro Gly Lys Met Glu Phe
```

```
    475                 480                 485

CAC CTC ATC CCC CAC TCG CTG CCC GGC TTC CCT GAT ACC CAG ACC ATC      2018
His Leu Ile Pro His S r Leu Pro Gly Phe Pro Asp Thr Gln Thr Ile
490                 495                 500                 505

CGC ATC GTC TAT GAC ATC CCC ACA GGC ATC CAG GGC CCT GAG CAC CCC      2066
Arg Ile Val Tyr Asp Ile Pro Thr Gly Ile Gln Gly Pro Glu His Pro
                510                 515                 520

AAC CCC GGG AAG AAG TTC ACC GCA AGA GGA TTC CCT CGC CAC TGC TAT      2114
Asn Pro Gly Lys Lys Phe Thr Ala Arg Gly Phe Pro Arg His Cys Tyr
            525                 530                 535

CTA CCC AAC AAC GAG AAA GGC CGG AAG GTG CTG CGG CTG CTC ATC ACG      2162
Leu Pro Asn Asn Glu Lys Gly Arg Lys Val Leu Arg Leu Leu Ile Thr
        540                 545                 550

GCC TGG GAG AGA AGA CTC ATC TTC ACT ATC GGC ACG TCC AAC ACC ACG      2210
Ala Trp Glu Arg Arg Leu Ile Phe Thr Ile Gly Thr Ser Asn Thr Thr
    555                 560                 565

GGC GAG TCG GAC ACC GTG GTG TGG AAC GAG ATC CAC CAC AAG ACC GAG      2258
Gly Glu Ser Asp Thr Val Val Trp Asn Glu Ile His His Lys Thr Glu
570                 575                 580                 585

TTT GGA TCC AAC CTC ACG GGA CAC GGC TAC CCG GAC GCT AGC TAC CTA      2306
Phe Gly Ser Asn Leu Thr Gly His Gly Tyr Pro Asp Ala Ser Tyr Leu
                590                 595                 600

GAC AAC GTG CTG GCT GAG CTC ACA GSC CAG GGC GTA TCC GAG GCT GCA      2354
Asp Asn Val Leu Ala Glu Leu Thr Xaa Gln Gly Val Ser Glu Ala Ala
            605                 610                 615

GGC AAG GCT TGAGGSCCAA GGCTGCCCAC CTTCCCTCCT GSTTTGGCCC             2403
Gly Lys Ala
        620

TGGTCCGGCA AATGCCTCCT TCGCCAGGTG TGTCCTGGTA GCCCAGGTTC AGGGCTGGGG    2463

AGGAGCCTGC GGAAGGGGCC GCAGCCATTC AGGGGACTGN CTGGNGGAAG TTGGATGAGG    2523

AGAGNTGGAT TTNAGGTTGG CCCC                                           2547
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 620 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Ser Arg Pro Gly His Gly Gly Leu Met Pro Val Asn Gly Leu Gly
 1               5                  10                  15

Phe Pro Pro Gln Asn Val Ala Arg Val Val Val Trp Glu Cys Leu Asn
            20                  25                  30

Glu His Ser Arg Trp Arg Pro Tyr Thr Ala Thr Val Cys His His Ile
        35                  40                  45

Glu Asn Val Leu Lys Glu Asp Ala Arg Gly Ser Val Val Leu Gly Gln
    50                  55                  60

Val Asp Ala Gln Leu Val Pro Tyr Ile Ile Asp Leu Gln Ser Met His
65                  70                  75                  80
```

Gln Phe Arg Gln Asp Thr Gly Thr Met Arg Pro Val Arg Arg Asn Phe
85 90 95

Tyr Asp Pro Ser Ser Ala Pro Gly Lys Gly Ile Val Trp Glu Trp Glu
100 105 110

Asn Asp Gly Gly Ala Trp Thr Ala Tyr Asp Met Asp Ile Cys Ile Thr
115 120 125

Ile Gln Asn Ala Tyr Glu Lys Gln His Pro Trp Leu Asp Leu Ser Ser
130 135 140

Leu Gly Phe Cys Tyr Leu Ile Tyr Phe Asn Ser Met Ser Gln Met Xaa
145 150 155 160

Arg Gln Thr Arg Arg Arg Arg Arg Leu Arg Arg Arg Leu Asp Leu Ala
165 170 175

Tyr Pro Leu Thr Val Gly Ser Ile Pro Lys Ser Gln Ser Trp Pro Val
180 185 190

Gly Xaa Ser Ser Gly Gln Pro Cys Ser Xaa Gln Gln Cys Leu Leu Val
195 200 205

Asn Ser Thr Arg Ala Val Ser Asn Val Ile Leu Xaa Ser Gln Arg Arg
210 215 220

Lys Val Xaa Pro Ala Pro Pro Leu Ser Xaa Pro Xaa Xaa Pro Gly Gly
225 230 235 240

Pro Pro Gly Ala Leu Gly Val Arg Pro Ser Val Thr Phe Thr Gly Xaa
245 250 255

Xaa Leu Xaa Glu Val Xaa Phe Xaa Gly Pro Val Glu Pro Xaa Xaa Ser
260 265 270

Pro Gly Xaa Pro Pro Arg Ser Pro Gly Ala Pro Gly Gly Ala Arg Thr
275 280 285

Pro Gly Gln Asn Asn Leu Asn Arg Xaa Gly Pro Gln Arg Thr Thr Xaa
290 295 300

Val Ser Ala Arg Ala Ser Ile Pro Pro Gly Val Pro Ala Leu Pro Val
305 310 315 320

Lys Asn Leu Asn Gly Thr Gly Pro Val His Pro Ala Leu Ala Gly Met
325 330 335

Thr Gly Ile Leu Leu Cys Ala Ala Gly Leu Pro Val Cys Leu Thr Arg
340 345 350

Ala Pro Lys Pro Ile Leu His Pro Pro Pro Val Ser Lys Ser Asp Val
355 360 365

Lys Pro Val Pro Gly Val Pro Gly Val Cys Arg Lys Thr Lys Lys Lys
370 375 380

His Leu Lys Lys Ser Lys Asn Pro Glu Asp Val Val Arg Arg Tyr Met
385 390 395 400

Gln Lys Val Lys Asn Pro Pro Asp Glu Asp Cys Thr Ile Cys Met Glu
405 410 415

Arg Leu Val Thr Ala Ser Gly Tyr Glu Gly Val Leu Arg His Lys Gly
420 425 430

Val Arg Pro Glu Leu Val Gly Arg Leu Gly Arg Cys Gly His Met Tyr
435 440 445

His Leu Leu Cys Leu Val Ala Met Tyr Ser Asn Gly Asn Lys Asp Gly

```
     450                 455                 460

Ser Leu Gln Cys Pro Thr Cys Lys Ala Ile Tyr Gly Glu Lys Thr Gly
465                 470                 475                 480

Thr Gln Pro Pro Gly Lys Met Glu Phe His Leu Ile Pro His Ser Leu
                485                 490                 495

Pro Gly Phe Pro Asp Thr Gln Thr Ile Arg Ile Val Tyr Asp Ile Pro
            500                 505                 510

Thr Gly Ile Gln Gly Pro Glu His Pro Asn Pro Gly Lys Lys Phe Thr
        515                 520                 525

Ala Arg Gly Phe Pro Arg His Cys Tyr Leu Pro Asn Asn Glu Lys Gly
    530                 535                 540

Arg Lys Val Leu Arg Leu Leu Ile Thr Ala Trp Glu Arg Arg Leu Ile
545                 550                 555                 560

Phe Thr Ile Gly Thr Ser Asn Thr Thr Gly Glu Ser Asp Thr Val Val
                565                 570                 575

Trp Asn Glu Ile His His Lys Thr Glu Phe Gly Ser Asn Leu Thr Gly
            580                 585                 590

His Gly Tyr Pro Asp Ala Ser Tyr Leu Asp Asn Val Leu Ala Glu Leu
        595                 600                 605

Thr Xaa Gln Gly Val Ser Glu Ala Ala Gly Lys Ala
    610                 615                 620
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 303 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Met Ala Ser Ser Ala Gly Ser Ala Ala Ser Gly Ser Val Val Pro Gly
1               5                   10                  15

Gly Gly Gly Ser Ala Ala Ser Ser Cys Ala Thr Met Ala Leu Ser Thr
            20                  25                  30

Ala Gly Ser Gly Gly Pro Pro Val Asn His Ala His Ala Val Cys Val
        35                  40                  45

Trp Glu Phe Glu Ser Arg Gly Lys Trp Leu Pro Tyr Ser Pro Ala Val
    50                  55                  60

Ser Gln His Leu Glu Arg Ala His Ala Lys Lys Leu Thr Arg Val Met
65                  70                  75                  80

Leu Ser Asp Ala Asp Pro Ser Leu Glu Gln Tyr Tyr Val Asn Val Arg
                85                  90                  95

Thr Met Thr Gln Glu Ser Glu Ala Glu Thr Arg Ser Gly Leu Leu Thr
            100                 105                 110

Ile Gly Val Arg Arg Met Leu Tyr Ala Pro Ser Ser Pro Ala Gly Lys
        115                 120                 125
```

```
Gly Thr Lys Trp Glu Trp Ser Gly Gly Ser Ala Asp Ser Asn Asn Asp
    130                 135                 140

Trp Arg Pro Tyr Asn Met His Val Gln Cys Ile Ile Glu Asp Ala Trp
145                 150                 155                 160

Ala Arg Gly Glu Gln Thr Leu Asp Leu Cys Asn Thr His Ile Gly Leu
                165                 170                 175

Pro Tyr Thr Ile Asn Phe Cys Asn Leu Thr His Val Arg Gln Pro Ser
            180                 185                 190

Gly Pro Met Arg Ser Ile Arg Arg Thr Gln Gln Ala Pro Tyr Pro Leu
        195                 200                 205

Val Lys Leu Thr Pro Gln Gln Ala Asn Gln Leu Lys Ser Asn Ser Ala
    210                 215                 220

Ser Val Ser Ser Gln Tyr Asn Thr Leu Pro Lys Leu Gly Asp Thr Lys
225                 230                 235                 240

Ser Leu His Arg Val Pro Met Thr Arg Gln Gln His Pro Leu Pro Thr
                245                 250                 255

Ser His Gln Val Gln Gln Gln Gln His Gln Leu Gln His Gln Gln Gln
            260                 265                 270

Gln Gln Gln Gln His His His Gln His Gln Gln Gln Gln His Gln Gln
        275                 280                 285

Gln Gln Gln His Gln Met Gln His His Gln Ile His His Gln Thr
    290                 295                 300
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Arg Lys Pro Pro Lys Lys His Ser Glu Ile Ser Thr Thr Asn Leu Arg
1               5                   10                  15

Gln Ile Leu Asn Asn Leu Asn Ile Phe Ser Ser Ser Thr Lys His Gln
            20                  25                  30

Ser Asn Met Ser Thr Ala Ala Ser Ala Ser Ser Ser Ser Ser Ser Ala
        35                  40                  45

Ser Leu His His Ala Asn His Leu Ser His Ala His Phe Ser His Ala
    50                  55                  60

Lys Asn Met Leu Thr Ala Ser Met Asn Ser His His Ser Arg Cys Ser
65                  70                  75                  80

Glu Gly Ser Leu Gln Ser Gln Arg Ser Ser Arg Met Gly Ser His Arg
                85                  90                  95

Ser Arg Ser Arg Thr Arg Thr Ser Asp Thr Asp Thr Asn Ser Val Lys
            100                 105                 110

Ser His Arg Arg Arg Pro Ser Val Asp Thr Val Ser Thr Tyr Leu Ser
        115                 120                 125
```

His Glu Ser Lys Glu Ser Leu Arg Ser Arg Asn Phe Ala Ile Ser Val
130 135 140

Asn Asp Leu Leu Asp Cys Ser Leu Gly Ser Asp Glu Val Phe Val Pro
145 150 155 160

Ser Val Pro Pro Ser Ser Leu Gly Glu Arg Ala Pro Val Pro Pro Pro
165 170 175

Leu Pro Leu His Pro
180

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 224 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ser Ile Ala Gly Ser Ile Val Gly Val Asp Pro Ala Ser Asp Met Ile
1 5 10 15

Ser Arg Phe Val Lys Val Val Glu Pro Pro Leu Trp Pro Asn Ala Gln
20 25 30

Pro Cys Pro Met Cys Met Glu Glu Leu Val His Ser Ala Gln Asn Pro
35 40 45

Ala Ile Ser Leu Ser Arg Cys Gln His Leu Met His Leu Gln Cys Leu
50 55 60

Asn Gly Met Ile Ile Ala Gln Gln Asn Glu Met Asn Lys Asn Leu Phe
65 70 75 80

Ile Glu Cys Pro Val Cys Gly Ile Val Tyr Gly Glu Lys Val Gly Asn
85 90 95

Gln Pro Ile Gly Ser Met Ser Trp Ser Ile Ile Ser Lys Asn Leu Pro
100 105 110

Gly His Glu Gly Gln Asn Thr Ile Gln Ile Val Tyr Asp Ile Ala Ser
115 120 125

Gly Leu Gln Thr Glu Glu His Pro His Pro Gly Arg Ala Phe Phe Ala
130 135 140

Val Gly Phe Pro Arg Ile Cys Tyr Leu Pro Asp Cys Pro Leu Gly Arg
145 150 155 160

Lys Val Leu Arg Phe Leu Lys Ile Ala Phe Asp Arg Arg Leu Leu Phe
165 170 175

Ser Ile Gly Arg Ser Val Thr Thr Gly Arg Glu Asp Val Val Ile Trp
180 185 190

Asn Ser Val Asp His Lys Thr Gln Phe Asn Met Phe Pro Asp Pro Thr
195 200 205

Tyr Leu Gln Arg Thr Met Gln Gln Leu Val His Leu Gly Val Thr Asp
210 215 220

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 204 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Ala Ser Ser Ala Gly Ser Ala Ala Ser Gly Ser Val Val Pro Gly
1 5 10 15

Gly Gly Gly Ser Ala Ala Ser Ser Cys Ala Thr Met Ala Leu Ser Thr
20 25 30

Ala Gly Ser Gly Gly Pro Pro Val Asn His Ala His Ala Val Cys Val
35 40 45

Trp Glu Phe Glu Ser Arg Gly Lys Trp Leu Pro Tyr Ser Pro Ala Val
50 55 60

Ser Gln His Leu Glu Arg Ala His Ala Lys Lys Leu Thr Arg Val Met
65 70 75 80

Leu Ser Asp Ala Asp Pro Ser Leu Glu Gln Tyr Tyr Val Asn Val Arg
85 90 95

Thr Met Thr Gln Glu Ser Glu Ala Glu Thr Arg Ser Gly Leu Leu Thr
100 105 110

Ile Gly Val Arg Arg Met Leu Tyr Ala Pro Ser Ser Pro Ala Gly Lys
115 120 125

Gly Thr Lys Trp Glu Trp Ser Gly Gly Ser Ala Asp Ser Asn Asn Asp
130 135 140

Trp Arg Pro Tyr Asn Met His Val Gln Cys Ile Ile Glu Asp Ala Trp
145 150 155 160

Ala Arg Gly Glu Gln Thr Leu Asp Leu Cys Asn Thr His Ile Gly Leu
165 170 175

Pro Tyr Thr Ile Asn Phe Cys Asn Leu Thr His Val Arg Gln Pro Ser
180 185 190

Gly Pro Met Arg Ser Ile Arg Arg Thr Gln Gln Ala
195 200

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Val Xaa Pro Ala Pro Pro Leu Ser Xaa Pro Xaa Xaa Pro Gly Gly Pro
1 5 10 15

Pro Gly Ala

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Xaa Ser Pro Gly Xaa Pro Pro Arg Ser Pro Gly Ala Pro Gly Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ser Ile Pro Pro Gly Val Pro Ala Leu Pro Val Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Arg Ala Pro Lys Pro Ile Leu His Pro Pro Pro Val Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Val Lys Pro Val Pro Gly Val Pro Gly Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Arg Ala Pro Val Pro Pro Pro Leu Pro Leu His Pro Arg Gln Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Arg Ala Pro Thr Met Pro Pro Pro Leu Pro Pro Val Pro Pro Gln Pro
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Arg Ala Val Pro Pro Pro Leu Pro Pro Arg Arg Lys Glu Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 61 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Cys Thr Ile Cys Met Glu Arg Leu Val Thr Ala Ser Gly Tyr Glu Gly
1 5 10 15

Val Leu Arg His Lys Gly Val Arg Pro Glu Leu Val Gly Arg Leu Gly
20 25 30

Arg Cys Gly His Met Tyr His Leu Leu Cys Leu Val Ala Met Tyr Ser
35 40 45

Asn Gly Asn Lys Asp Gly Ser Leu Gln Cys Pro Thr Cys
50 55 60

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CACCATCTGC ATGGAGCGAC TGGT 24

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TGTGGCCACA TGTACCACCT GCTG 24

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TACGGGGAGA AGACGGGTAC GCAG 24

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AAGATGGAGT TCCACCTCAT CCC 23

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Cys Met Glu Arg Leu Val
1 5

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Tyr Gly Glu Lys Thr Gly
1 5

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Thr Ile Arg Ile Val Tyr Asp Ile
1 5

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Gly Phe Pro Arg His Cys Tyr Leu Pro

1 5

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Arg Arg Leu Ile Phe Thr Ile Gly
1 5

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Met Cys Met Glu Glu Leu Val
1 5

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Tyr Gly Glu Lys Val Gly
1 5

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Thr Ile Gln Ile Val Tyr Asp Ile
1 5

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Gly Phe Pro Arg Ile Cys Tyr Leu Pro
1 5

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Arg Arg Leu Leu Phe Ser Ile Gly
1 5

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 69, lines 1-20 of the description

A. IDENTIFICATION OF DEPOSIT *

Further deposits are identified on an additional sheet *

Name of depositary institution *

American Type Culture Collection

Address of depositary institution (including postal code and country) *

12301 Parklawn Drive
Rockville, MD 20852
US

Date of deposit * November 17, 1995 Accession Number * 97341

B. ADDITIONAL INDICATIONS * (leave blank if not applicable). This information is continued on a separate attached sheet

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS * (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☒ This sheet was received with the international application when filed (to be checked by the receiving Office)

Gilda D. Barnes
(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau *

was

(Authorized Officer)

Form PCT/RO/134 (January 1981)

WHAT IS CLAIMED IS:

1. A purified vertebrate Deltex protein.

2. The protein of claim 1 which is a mammalian protein.

3. The protein of claim 1 which is a human protein.

4. The protein of claim 1 having the amino acid sequence depicted in Figure 2A-C (SEQ ID NO:12).

5. A purified protein comprising a fragment of a vertebrate Deltex protein, said fragment consisting of at least 10 continuous amino acids of the vertebrate Deltex protein.

6. A purified protein comprising a fragment of a vertebrate Deltex protein, said fragment consisting of at least 20 continuous amino acids of the vertebrate Deltex protein.

7. A purified fragment of a vertebrate Deltex protein consisting of at least 10 continuous amino acids of a vertebrate Deltex protein, which displays one or more functional activities associated with a full-length vertebrate Deltex protein.

8. The fragment of claim 7 which consists of at least 20 continuous amino acids of the Deltex protein.

9. The protein of claim 5 in which the protein is able to be bound by an antibody to a Deltex protein.

10. A purified protein comprising a fragment of a vertebrate Deltex protein, which fragment binds to a Notch protein or to a molecule comprising the cdc10/SW16/ankyrin repeats of a Notch protein.

11. A purified protein comprising a fragment of a first vertebrate Deltex protein, which fragment binds to a second Deltex protein or to a molecule comprising a fragment of a second Deltex protein.

12. A purified protein comprising a fragment of a vertebrate Deltex protein, which fragment comprises a SH3-binding domain of the vertebrate Deltex protein.

13. A chimeric protein comprising a functionally active fragment of a vertebrate Deltex protein joined via a peptide bond to an amino acid sequence of a protein other than a vertebrate Deltex protein.

14. The protein of claim 13 in which the fragment binds to a Notch protein or to a molecule comprising the cdc10/SW16/ankyrin repeats of a Notch protein.

15. The protein of claim 13 in which the fragment comprises an SH3-binding domain.

16. The protein of claim 13 in which the fragment comprises a zinc finger domain.

17. A purified derivative of the protein of claim 1, which is characterized by the ability to be bound by antibody to the protein of claim 1, which derivative has one or more insertions, deletions, or substitutions relative to the protein.

18. A purified peptide having an amino acid sequence in the range of 10-35 amino acids, said sequence being a portion of a vertebrate Deltex protein sequence.

19. A purified derivative of the protein of claim 1, which is able to display one or more functional activities of the protein of claim 1.

20. A molecule comprising the sequence of a human Deltex protein.

21. An antibody which binds to a vertebrate Deltex protein, and which does not bind to a *Drosophila* Deltex protein.

22. The antibody of claim 21 which binds to a human Deltex protein.

23. The antibody of claim 21 which is monoclonal.

24. A fragment or derivative of the antibody of claim 23 containing the binding domain of the antibody.

25. A purified nucleic acid encoding a vertebrate Deltex protein.

26. The nucleic acid of claim 25 which lacks introns.

27. The nucleic acid of claim 25 which encodes a protein having the amino acid sequence depicted in Figure 2A-C (SEQ ID NO:12).

28. The nucleic acid of claim 25 which comprises the coding region of the nucleotide sequence depicted in Figure 2A-C (part of SEQ ID NO:11).

29. A purified nucleic acid complementary to the nucleic acid of claim 25.

30. The nucleic acid of claim 25 which encodes a human Deltex protein.

31. A purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of low stringency, said second nucleic acid comprising the nucleotide sequence depicted in Figure 2A-C (SEQ ID NO:11), said first nucleic acid comprising at least 110 continuous nucleotides of SEQ ID NO:11.

32. A purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of high stringency, said second nucleic acid comprising the nucleotide sequence depicted in Figure 2A-C (SEQ ID NO:11), said first nucleic acid comprising at least 110 continuous nucleotides of SEQ ID NO:11.

33. A purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of low stringency, said second nucleic acid encoding a protein comprising the amino acid sequence depicted in Figure 2A-C (SEQ ID NO:12), said first nucleic acid encoding a protein comprising the first 50 amino acids of SEQ ID NO:12.

34. A purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of high stringency, said second nucleic acid encoding a protein comprising the amino acid sequence depicted in Figure 2A-C (SEQ ID NO:12), said first nucleic acid encoding a protein comprising the first 50 amino acids of SEQ ID NO:12.

35. A purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of high stringency, said second nucleic acid comprising nucleotide numbers 500-1044, 1045-1821, or 1822-2380 of SEQ ID NO:11.

36. A purified first nucleic acid which is hybridizable to a second nucleic acid under conditions of low stringency, said second nucleic acid comprising nucleic acids 500-1044, 1045-1821, or 1822-2370 of SEQ ID NO:11.

37. A purified nucleic acid comprising a nucleotide sequence encoding a protein comprising the first 25 amino acids of SEQ ID NO:12.

38. A purified nucleic acid comprising a nucleotide sequence encoding a protein comprising the first 50 amino acids of SEQ ID NO:12.

39. A purified nucleic acid comprising a nucleotide sequence encoding a protein comprising the first 100 amino acids of SEQ ID NO:12.

40. A purified nucleic acid comprising a nucleotide sequence encoding a protein comprising the first 150 amino acids of SEQ ID NO:12.

41. A purified nucleic acid comprising a nucleotide sequence encoding a protein comprising the first 230 amino acids of SEQ ID NO:12.

42. A purified nucleic acid comprising 110 continuous nucleotides of SEQ ID NO:11.

43. A purified nucleic acid encoding the protein of claim 5.

44. A purified nucleic acid encoding the protein of claim 10.

45. A purified nucleic acid encoding the protein of claim 11.

46. A purified nucleic acid encoding the protein of claim 12

47. A nucleic acid encoding the chimeric protein of claim 13.

48. The nucleic acid of claim 25 as contained in plasmid pBS hdx as deposited with the ATCC and assigned accession number 97341.

49. A nucleic acid vector comprising the nucleic acid of claim 25.

50. A nucleic acid vector comprising the nucleic acid of claim 26.

51. A recombinant cell containing the nucleic acid vector of claim 49.

52. A recombinant cell containing the nucleic acid vector of claim 50.

53. A method for producing a vertebrate Deltex protein comprising growing the recombinant cell of claim 51, such that the vertebrate Deltex protein is expressed by the cell; and recovering the expressed vertebrate Deltex protein.

54. A method for producing a protein comprising growing a cell containing a recombinant nucleic acid comprising the nucleic acid of claim 33, such that the protein is expressed by the cell; and recovering the expressed protein.

55. A pharmaceutical composition comprising a therapeutically effective amount of a vertebrate Deltex protein; and a pharmaceutically acceptable carrier.

56. The composition of claim 55 in which the vertebrate Deltex protein is a human Deltex protein.

57. A pharmaceutical composition comprising a therapeutically effective amount of the protein of claim 7; and a pharmaceutically acceptable carrier.

58. A pharmaceutical composition comprising a therapeutically effective amount of the protein of claim 10; and a pharmaceutically acceptable carrier.

59. A pharmaceutical composition comprising a therapeutically effective amount of the protein of claim 11; and a pharmaceutically acceptable carrier.

60. A pharmaceutical composition comprising a therapeutically effective amount of the protein of claim 12; and a pharmaceutically acceptable carrier.

61. A pharmaceutical composition comprising a therapeutically effective amount of the protein of claim 13; and a pharmaceutically acceptable carrier.

62. A pharmaceutical composition comprising a therapeutically effective amount of a derivative of a vertebrate Deltex protein, which derivative is characterized by the ability to bind to a Notch protein or to a molecule comprising the cdc10/SW16/ankyrin repeats of a Notch protein.

63. A pharmaceutical composition comprising a therapeutically effective amount of a nucleic acid encoding a vertebrate Deltex protein; and a pharmaceutically acceptable carrier.

64. The pharmaceutical composition of claim 63 in which the Deltex protein is a human Deltex protein.

65. A pharmaceutical composition comprising a therapeutically effective amount of an antibody which binds to a vertebrate Deltex protein but not to a *Drosophila* Deltex protein, or a fragment or derivative of the antibody containing the binding domain thereof; and a pharmaceutically acceptable carrier.

66. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in need of such treatment or prevention a therapeutically effective amount of a molecule which antagonizes the function of a vertebrate Deltex protein.

67. The method according to claim 66 in which the disease or disorder is a malignancy characterized by increased Notch activity or increased

expression of a Notch protein or of a Notch derivative capable of being bound by an anti-Notch antibody, relative to said Notch activity or expression in an analogous non-malignant sample.

68. The method according to claim 66 in which the disease or disorder is cervical cancer.

69. The method according to claim 66 in which the disease or disorder is breast cancer.

70. The method according to claim 66 in which the disease or disorder is colon cancer.

71. The method according to claim 66 in which the malignancy is selected from the group consisting of melanoma, seminoma, and lung cancer.

72. The method according to claim 67 in which the subject is a human.

73. The method according to claim 66 in which the molecule is an antibody to vertebrate Deltex or a derivative of said antibody containing the binding domain thereof, which antibody does not bind to *Drosophila* Deltex.

74. The method according to claim 66 in which the molecule is a protein comprising a portion of a vertebrate Deltex protein capable of binding to a Notch protein or to a second molecule comprising the cdc10/SW16/ankyrin repeats of a Notch protein.

75. The method according to claim 66 in which the molecule is a protein comprising the SH3 binding domain of a vertebrate Deltex protein.

76. The method according to claim 66 in which the molecule is a protein comprising the zinc finger domain of a vertebrate Deltex protein.

77. The method according to claim 66 in which the molecule is an oligonucleotide which (a) consists of at least six nucleotides; (b) comprises a sequence complementary to at least a portion of an RNA transcript of a vertebrate *deltex* gene; and (c) is hybridizable to the RNA transcript.

78. A method of treating or preventing a disease or disorder in a subject in need of such treatment or prevention comprising administering to the subject a therapeutically effective amount of a molecule which promotes the function of a vertebrate Deltex protein.

79. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of a vertebrate Deltex protein.

80. The method according to claim 79 in which the Deltex protein is a human Deltex protein.

81. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of the nucleic acid of claim 25.

82. A method of treating or preventing a malignancy in a subject comprising administering to a subject in need of such treatment or prevention an effective amount of the antibody of claim 21.

83. A method for treating a patient with a tumor, of a tumor type characterized by expression of a *Notch* or vertebrate *deltex* gene, comprising administering to the patient an effective amount of an oligonucleotide, which oligonucleotide (a) consists of at least six nucleotides; (b) comprises a sequence

complementary to at least a portion of an RNA transcript of the vertebrate *deltex* gene; and (c) is hybridizable to the RNA transcript.

84. An isolated oligonucleotide consisting of at least six nucleotides, and comprising a sequence complementary to at least a portion of an RNA transcript of a vertebrate *deltex* gene, which oligonucleotide is hybridizable to the RNA transcript.

85. A pharmaceutical composition comprising the oligonucleotide of claim 84; and a pharmaceutically acceptable carrier.

86. A method of inhibiting the expression of a nucleic acid sequence encoding a vertebrate Deltex protein in a cell comprising providing the cell with an effective amount of the oligonucleotide of claim 84.

87. A method of diagnosing a disease or disorder characterized by an aberrant level of Notch-vertebrate Deltex protein binding activity in a patient, comprising measuring the ability of a Notch protein in a sample derived from the patient to bind to a vertebrate Deltex protein, in which an increase or decrease in the ability of the Notch protein to bind to the vertebrate Deltex protein, relative to the ability found in an analogous sample from a normal individual, indicates the presence of the disease or disorder in the patient.

88. A method of identifying a molecule that inhibits or reduces the binding of a vertebrate Deltex protein to a Notch protein, comprising:

(a) contacting (i) a Notch protein or fragment thereof that mediates binding to a Deltex protein, and (ii) a vertebrate Deltex protein or fragment thereof that mediates binding to a Notch protein, such that binding between the Notch protein or fragment and the Deltex protein or fragment can occur, in the presence of one or more molecules which are desired to be tested for the ability to inhibit or reduce binding between

the Notch protein or fragment and the Deltex protein or fragment; and

(b) identifying the one or more molecules that inhibit or reduce the binding of the Deltex protein or fragment to the Notch protein or fragment.

89. The method of claim 88 in which the Deltex protein is a human Deltex protein.

90. A method of inactivating Notch function in a cell comprising introducing into the cell a molecule, said molecule comprising (a) a Deltex protein or portion thereof that mediates binding to a Notch protein; and (b) a protease or proteolytically active portion thereof.

91. A method for the expansion of a precursor cell comprising contacting the cell with an amount of a vertebrate Deltex portion or functionally active portion thereof effective to inhibit differentiation of the cell, and exposing the cell to cell growth conditions such that the cell proliferates.

```
          10         20         30         40         50         60
      *    *     *    *     *    *     *    *     *    *     *    *
AAATGCTAGA AAAACCGTTT TTACCATCAA ACGTGAATTC TTAAGCTGCG CCTAAACGAA

          70         80         90        100        110        120
      *    *     *    *     *    *     *    *     *    *     *    *
ACCGAGTGAC TAAAGAACCA GAACGAAAAC TTCGGGAAAA TGGAAGCCAG GGAAAATCAG

         130        140        150        160        170        180
      *    *     *    *     *    *     *    *     *    *     *    *
GGATAACTAA CGCTGGCAGC GGGTCCACCA TTTTTAATTT CTTTGTTTAT TTTGTGCCCA

         190        200        210        220        230        240
      *    *     *    *     *    *     *    *     *    *     *    *
TCTTCGCGAG CGAGCGAGAT AGCGCGACAG CAACAGCAAG AGAGAGCGAG AGAGAGAGTG

         250        260        270        280        290        300
      *    *     *    *     *    *     *    *     *    *     *    *
AGTGAGTGAG AGCTAGTGAA GAGAGCGCAG GAGGAGTTGG ATATGGAAAT GGGCATGGAT

         310        320        330        340        350        360
      *    *     *    *     *    *     *    *     *    *     *    *
ATGGCAATGG GCTCACTCCA CGGATAACGG ATCAACTGCA AGCAATGGCC AGCAGCGCCG
                                                M  A    S  S  A>
```

FIG. 1A

```
          370         380         390         400         410         420
      *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GAAGTGCGGC ATCCGGATCC GTTGTTCCCG GTGGCGGAGG TAGCGCCGCC TCCAGTTGTG
G  S  A  A   S  G  S   V  V  P   G  G  G  G   S  A  A   S  S  C>

          430         440         450         460         470         480
      *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CCACCATGGC CCTGTCCACC GCCGGATCCG GTGGGCCGCC CGTGAACCAC GCCCACGCCG
A  T  M  A   L  S  T   A  G  S   G  G  P  P   V  N  H   A  H  A>

          490         500         510         520         530         540
      *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TCTGCGTGTG GGAGTTCGAG TCGCGCGGCA AGTGGCTGCC CTATTCGCCG GCGGTGTCGC
V  C  V  W   E  F  E   S  R  G   K  W  L  P   Y  S  P   A  V  S>

          550         560         570         580         590         600
      *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AGCACTTGGA ACGCGCCCAC GCCAAGAAAC TGACGCGCGT CATGCTGAGC GATGCGGATC
Q  H  L  E   R  A  H   A  K  K   L  T  R  V   M  L  S   D  A  D>

          610         620         630         640         650         660
      *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CCAGCCTGGA GCAGTACTAC GTCAACGTGC GCACAATGAC CCAGGAATCG GAGGCGGAAA
P  S  L  E   Q  Y  Y   V  N  V   R  T  M  T   Q  E  S   E  A  E>
```

FIG. 1B

```
          670         680         690         700         710         720
      *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CGCGCTCCGG CCTGCTGACC ATCGGTGTTC GGCGCATGTT ATACGCACCC AGCTCGCCGG
T  R  S  G   L  L  T   I  G  V   R  R  M  L   Y  A  P   S  S  P>

          730         740         750         760         770         780
      *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CGGGCAAGGG CACCAAGTGG GAGTGGTCGG GCGGCAGTGC CGATAGCAAC AACGACTGGC
A  G  K  G   T  K  W   E  W  S   G  G  S  A   D  S  N   N  D  W>

          790         800         810         820         830         840
      *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
GGCCCTACAA CATGCACGTC CAGTGCATCA TCGAGGACGC CTGGGCGAGG GGCGAACAAA
R  P  Y  N   M  H  V   Q  C  I   I  E  D  A   W  A  R   G  E  Q>

          850         860         870         880         890         900
      *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CCTTGGACCT GTGCAACACC CACATCGGCC TGCCGTACAC CATTAATTTT TGCAATCTCA
T  L  D  L   C  N  T   H  I  G   L  P  Y  T   I  N  F   C  N  L>

          910         920         930         940         950         960
      *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CCCACGTGCG CCAACCCAGC GGACCCATGC GCAGCATTCG GCGTACCCAA CAGGCGCCGT
T  H  V  R   Q  P  S   G  P  M   R  S  I  R   R  T  Q   Q  A  P>
```

FIG. 1C

```
       970         980         990        1000        1010        1020
     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
ATCCCTTGGT GAAACTAACG CCACAACAGG CCAACCAACT CAAGTCGAAT TCCGCCAGCG
Y  P  L  V   K  L  T   P  Q  Q   A  N  Q  L   K  S  N   S  A  S>

       1030        1040        1050        1060        1070        1080
     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
TGAGCAGCCA GTACAACACT CTACCCAAAC TGGGCGACAC CAAGAGCCTG CACAGAGTGC
V  S  S  Q   Y  N  T   L  P  K   L  G  D  T   K  S  L   H  R  V>

       1090        1100        1110        1120        1130        1140
     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
CCATGACCAG GCAACAGCAC CCATTGCCCA CCAGCCATCA AGTGCAGCAG CAGCAGCATC
P  M  T  R   Q  Q  H   P  L  P   T  S  H  Q   V  Q  Q   Q  Q  H>

       1150        1160        1170        1180        1190        1200
     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
AGCTCCAGCA TCAACAGCAG CAGCAGCAGC AACATCATCA CCAGCATCAG CAACAACAGC
Q  L  Q  H   Q  Q  Q   Q  Q  Q   Q  H  H  H   Q  H  Q   Q  Q  Q>

       1210        1220        1230        1240        1250        1260
     *   *   *   *   *   *   *   *   *   *   *   *   *   *   *   *
ATCAGCAACA GCAGCAACAT CAGATGCAGC ACCATCAGAT CCATCATCAG ACGGCGCCCA
H  Q  Q  Q   Q  Q  H   Q  M  Q   H  H  Q  I   H  H  Q   T  A  P>
```

FIG. 1D

```
      1270        1280        1290        1300        1310        1320
       *   *       *   *       *   *       *   *       *   *       *   *
GGAAGCCGCC CAAGAAGCAC AGCGAGATCT CCACCACCAA TCTACGCCAG ATACTCAACA
R  K  P  P  K  K  H  S  E  I  S  T  T  N  L  R  Q  I  L  N>

      1330        1340        1350        1360        1370        1380
       *   *       *   *       *   *       *   *       *   *       *   *
ACCTAAACAT CTTCAGCAGC AGCACTAAGC ACCAATCGAA CATGTCGACG GCGGCCAGTG
N  L  N  I  F  S  S  S  T  K  H  Q  S  N  M  S  T  A  A  S>

      1390        1400        1410        1420        1430        1440
       *   *       *   *       *   *       *   *       *   *       *   *
CCAGTTCATC CTCCTCATCG GCCTCGCTGC ACCATGCCAA CCATCTGTCG CATGCGCACT
A  S  S  S  S  S  S  A  S  L  H  H  A  N  H  L  S  H  A  H>

      1450        1460        1470        1480        1490        1500
       *   *       *   *       *   *       *   *       *   *       *   *
TTTCGCACGC CAAGAACATG CTGACTGCCT CGATGAACAG TCATCATAGT CGCTGCTCGG
F  S  H  A  K  N  M  L  T  A  S  M  N  S  H  H  S  R  C  S>

      1510        1520        1530        1540        1550        1560
       *   *       *   *       *   *       *   *       *   *       *   *
AGGGATCGCT GCAGTCGCAA AGGAGCAGCC GGATGGGCTC GCATCGCTCG AGATCGCGAA
E  G  S  L  Q  S  Q  R  S  S  R  M  G  S  H  R  S  R  S  R>
```

FIG. 1E

```
      1570        1580        1590        1600        1610        1620
       *   *       *   *       *   *       *   *       *   *       *   *
CGCGGACCTC GGACACGGAC ACGAACAGTG TGAAATCGCA TCGGCGGAGA CCCAGTGTGG
T  R  T  S   D  T  D   T  N  S   V  K  S  H   R  R  R   P  S  V>

      1630        1640        1650        1660        1670        1680
       *   *       *   *       *   *       *   *       *   *       *   *
ACACCGTGTC CACTTACCTC AGCCACGAGA GCAAGGAGAG CCTGCGCAGC AGGAACTTTG
D  T  V  S   T  Y  L   S  H  E   S  K  E  S   L  R  S   R  N  F>

      1690        1700        1710        1720        1730        1740
       *   *       *   *       *   *       *   *       *   *       *   *
CCATTTCGGT CAATGATCTG CTGGACTGCT CGCTTGGCAG CGATGAAGTT TTTGTGCCCT
A  I  S  V   N  D  L   L  D  C   S  L  G  S   D  E  V   F  V  P>

      1750        1760        1770        1780        1790        1800
       *   *       *   *       *   *       *   *       *   *       *   *
CCGTGCCGCC ATCGTCGCTG GGCGAAAGGG CGCCGGTGCC GCCGCCATTA CCACTGCATC
S  V  P  P   S  S  L   G  E  R   A  P  V  P   P  P  L   P  L  H>

      1810        1820        1830        1840        1850        1860
       *   *       *   *       *   *       *   *       *   *       *   *
CGCGACAGCA ACAGCAGCAG CAACAACAGC AGCAACAGCT GCAGATGCAA CAGCAGCAAC
P  R  Q  Q   Q  Q  Q   Q  Q  Q   Q  Q  Q  L   Q  M  Q   Q  Q  Q>
```

FIG. 1F

```
      1870        1880        1890        1900        1910        1920
       *   *       *   *       *   *       *   *       *   *       *   *
AGGCGCAGCA GCAGCAGCAG CAATCAATCG CCGGTTCGAT TGTGGGCGTG GACCCGGCCA
Q  A  Q  Q   Q  Q  Q   Q  S  I   A  G  S  I   V  G  V   D  P  A>

      1930        1940        1950        1960        1970        1980
       *   *       *   *       *   *       *   *       *   *       *   *
GCGATATGAT ATCGCGTTTT GTCAAGGTGG TGGAGCCACC GCTGTGGCCC AATGCCCAGC
S  D  M  I   S  R  F   V  K  V   V  E  P  P   L  W  P   N  A  Q>

      1990        2000        2010        2020        2030        2040
       *   *       *   *       *   *       *   *       *   *       *   *
CCTGTCCCAT GTGCATGGAG GAGCTGGTGC ACTCCGCCCA GAATCCGGCC ATTTCGCTGA
P  C  P  M   C  M  E   E  L  V   H  S  A  Q   N  P  A   I  S  L>

      2050        2060        2070        2080        2090        2100
       *   *       *   *       *   *       *   *       *   *       *   *
GTCGCTGCCA GCATCTCATG CATTTGCAGT GCCTCAATGG GATGATAATT GCCCAGCAAA
S  R  C  Q   H  L  M   H  L  Q   C  L  N  G   M  I  I   A  Q  Q>

      2110        2120        2130        2140        2150        2160
       *   *       *   *       *   *       *   *       *   *       *   *
ACGAAATGAA CAAGAACCTT TTCATCGAGT GCCCTGTATG CGGCATCGTT TACGGCGAGA
N  E  M  N   K  N  L   F  I  E   C  P  V  C   G  I  V   Y  G  E>
```

FIG. 1G

```
      2170        2180        2190        2200        2210        2220
       *   *       *   *       *   *       *   *       *   *       *   *
AGGTCGGCAA TCAGCCCATT GGCAGCATGT CGTGGAGCAT AATTAGCAAG AATCTGCCAG
K  V  G  N   Q  P  I   G  S  M   S  W  S  I   I  S  K   N  L  P>

      2230        2240        2250        2260        2270        2280
       *   *       *   *       *   *       *   *       *   *       *   *
GACACGAGGG TCAGAACACC ATACAGATTG TTTACGACAT TGCATCGGGA CTGCAGACGG
G  H  E  G   Q  N  T   I  Q  I   V  Y  D  I   A  S  G   L  Q  T>

      2290        2300        2310        2320        2330        2340
       *   *       *   *       *   *       *   *       *   *       *   *
AGGAGCATCC GCATCCAGGT CGTGCCTTCT TCGCCGTGGG ATTCCCGCGG ATCTGCTACT
E  E  H  P   H  P  G   R  A  F   F  A  V  G   F  P  R   I  C  Y>

      2350        2360        2370        2380        2390        2400
       *   *       *   *       *   *       *   *       *   *       *   *
TGCCGGACTG CCCGCTGGGG CGAAAGGTTT TGCGCTTCCT CAAGATTGCA TTCGATCGTC
L  P  D  C   P  L  G   R  K  V   L  R  F  L   K  I  A   F  D  R>

      2410        2420        2430        2440        2450        2460
       *   *       *   *       *   *       *   *       *   *       *   *
GGCTGCTTTT CTCGATCGGA CGATCGGTGA CCACCGGACG CGAGGATGTG GTGATCTGGA
R  L  L  F   S  I  G   R  S  V   T  T  G  R   E  D  V   V  I  W>
```

FIG. 1H

```
      2470        2480        2490        2500        2510        2520
       *   *       *   *       *   *       *   *       *   *       *   *
ACAGTGTGGA TCACAAGACG CAGTTCAATA TGTTTCCGGA TCCCACCTAT TTGCAGCGAA
N  S  V  D   H  K  T   Q  F  N   M  F  P  D   P  T  Y   L  Q  R>

      2530        2540        2550        2560        2570        2580
       *   *       *   *       *   *       *   *       *   *       *   *
CCATGCAACA GCTGGTGCAC CTGGGCGTGA CGGATTAAGG ATTAGTTCCC TGTCCCCAAG
T  M  Q  Q   L  V  H   L  G  V   T  D  *>

      2590        2600        2610        2620        2630        2640
       *   *       *   *       *   *       *   *       *   *       *   *
TAGAACTACC AACCAACCAA TCAACCACCC ACCCACCGAA GTCCCCTCGA TCATTCTCTT

      2650        2660        2670        2680        2690        2700
       *   *       *   *       *   *       *   *       *   *       *   *
CCATTCGTCG TTAAGTTACT TTCTACATAA TCTCAGTGTG TGTGCAATCC TCGTTTACTA

      2710        2720        2730        2740        2750        2760
       *   *       *   *       *   *       *   *       *   *       *   *
TGATATATTT TTTTTATAGA TATATTGTAA TAGCGTTCGA GCTGCTCGAA CCCTAAAACA

      2770        2780        2790        2800        2810        2820
       *   *       *   *       *   *       *   *       *   *       *   *
ACAGCAAACC ACAATTGCAA TTGTAGCTTC CTTTCCGCTC TTCCAATTCG TATTTGTACG
```

FIG. 1I

```
      2830        2840        2850        2860        2870        2880
       *   *       *   *       *   *       *   *       *   *       *   *
CACATACGCA ATAAGTTGGC GTACATCATA TGTATTAGCT AGTTAGTTAG TTAGTTAGTT

      2890        2900        2910        2920        2930        2940
       *   *       *   *       *   *       *   *       *   *       *   *
AGTTGTAGCT GTAGTTCCCA AGAGAATCTT GACCCAAGAC ACCTACTAGT ATTAGGCATT

      2950        2960        2970        2980        2990        3000
       *   *       *   *       *   *       *   *       *   *       *   *
ATCCTGATTC TTGATTCCTG ATTCGATTCA AGCCAAGCCA AGCCACGCCA TTCGAGTGCA

      3010        3020        3030        3040        3050        3060
       *   *       *   *       *   *       *   *       *   *       *   *
AGCTGTGCCA AAATCGTAGC GCTCCCGTTT ATAGGATATG TATATTGTTG ATATAGCTAG

      3070        3080        3090        3100        3110        3120
       *   *       *   *       *   *       *   *       *   *       *   *
CTATAACCAT TGCCCATCTC TCCATCTCTC TCGGTTTCGA ATTTGTCTCT TTCATCAGAT

      3130        3140        3150        3160        3170        3180
       *   *       *   *       *   *       *   *       *   *       *   *
CCATGTGAAT TTTCTTTATA TCGGATTTAT ATAGGATTAA AATAGTATTT TGAGAGAGGA
```

FIG. 1J

```
      3190        3200        3210        3220        3230        3240
       *   *       *   *       *   *       *   *       *   *       *   *
AATGGAGATG GGTAAATTCG ATAGACTTGT CTCACTTGTC TTGGCCATTT AATCTCTTTC

      3250        3260        3270        3280        3290        3300
       *   *       *   *       *   *       *   *       *   *       *   *
ATTCAGCGAA TTTGATGTGA TTTTAATTTG AATTATTCAT TATTAAACGG AGCATTTAGG

      3310        3320        3330        3340        3350        3360
       *   *       *   *       *   *       *   *       *   *       *   *
AAGCATAGTT GTAACGCAGC CAGATATTCC ATTACGCATA TACATATACA TATACATATA

      3370        3380        3390        3400        3410        3420
       *   *       *   *       *   *       *   *       *   *       *   *
CATACATACA TAAACATATT TTAACATAGC CCCATAGCCA TACGACATAA CAATAATTTT

      3430        3440        3450        3460        3470        3480
       *   *       *   *       *   *       *   *       *   *       *   *
TTTTATCGAA TCCCTTGCAT ACATTTGATG AATTGTTGCT TTCATATTGA TATCATCGAG

      3490        3500        3510        3520        3530        3540
       *   *       *   *       *   *       *   *       *   *       *   *
CATCGAACGA ACTATCGTAT ACATCGCCAA TATATAGCAT ATATAGCATA TAGTATGTAG
```

FIG. 1K

```
      3550        3560        3570        3580        3590        3600
       *   *       *   *       *   *       *   *       *   *       *   *
AGATCGTACG GACAGCTAGC GGCTACTGAC CGCGCCACCA TATTTGATAT GATATGATAT

      3610        3620        3630        3640        3650        3660
       *   *       *   *       *   *       *   *       *   *       *   *
GATTTTACTA AGTTGTATTT AGCACTGATT AGTTATTAAA GTTCATTTGA CGAATATTCC

      3670        3680        3690        3700        3710        3720
       *   *       *   *       *   *       *   *       *   *       *   *
ACAACAAATT CCACACCATT TATGTATGCA TATTACGCAT ATATAATACA GTACATTTAT

      3730        3740        3750        3760        3770
       *   *       *   *       *   *       *   *       *   *
ATATAGTTCA AATAAAGTAA CTTCATTCAT GTTCAAAAAA AAAAAAAAAA A
```

FIG. 1L

```
          10                  30                  50
GCGAGAAGCCCCACTGAAGCCGGGCGCAGGGTCTGGGACGCAGTTGGGAGTGCAAAGGGC

          70                  90                 110
TGGCTGAGAGCCGCAGGAGCAGCAGGCTGTGGCCCAGGCCTCCTGGGTGACAGGCCCTGT

         130                 150                 170
CTGGCGGGGAACAGGGACCAAGAGACAACACAGAAGAGGCTGGACCTCGAACAGGGGCGG

         190                 210                 230
CTGCCTCACTCCCTACCTGAGCCAGCCGAGGGGGCCAAGGACTTTAGAGCTGTTCCTCC

         250                 270                 290
GGCATAAGAGAGACACTTGCTTTCCAGGGCAGCACCCTTTATCGGAGAAGGCTCTACAGG

         310                 330                 350
GAAGGGGTCTTTGCAGCCTGGATGGCCATCCCACATTCCTTTAACGGAGGTCTCTAGGCC

         370                 390                 410
TCAGAGAGAACCCAGAGTTAGAAAGGAGGCCAGACGGTCCTTGCTGTCCCCCTGGGGAGA

         430                 450                 470
GAGGAAGTTGCCGCCTGCTGCCAGGCCCAGGAGGAGCTGGGCCTGCAATAGTGGGGGACC

         490                 510                 530
TGGCCCCTGAGGCAGTGGCGGCCATGTCACGGCCAGGCCACGGTGGGCTGATGCCTGTGA
                    M   S   R   P   G   H   G   G   L   M   P   V   N
```

FIG. 2A

```
       550                       570                       590
ATGGTCTGGGCTTCCCACCGCAGAACGTGGCCCGGGTGGTGGTGTGGGAGTGTCTGAATG
  G  L  G  F  P  P  Q  N  V  A  R  V  V  V  W  E  C  L  N  E

       610                       630                       650
AGCACAGCCGCTGGCGGCCCTACACGGCCACCGTGTGCCACCACATTGAGAACGTGCTGA
  H  S  R  W  R  P  Y  T  A  T  V  C  H  H  I  E  N  V  L  K

       670                       690                       710
AGGAGGACGCTCGCGGTTCCGTGGTCCTGGGGCAGGTGGACGCCCAGCTTGTGCCCTACA
  E  D  A  R  G  S  V  V  L  G  Q  V  D  A  Q  L  V  P  Y  I

       730                       750                       770
TCATCGACCTGCAGTCCATGCACCAGTTTCGCCAGGACACAGGCACCATGCGGCCCGTGC
  I  D  L  Q  S  M  H  Q  F  R  Q  D  T  G  T  M  R  P  V  R

       790                       810                       830
GGCGCAACTTCTACGACCCGTCGTCGGCGCCGGGCAAGGGCATCGTGTGGGAGTGGGAGA
  R  N  F  Y  D  P  S  S  A  P  G  K  G  I  V  W  E  W  E  N

       850                       870                       890
ACGACGGCGGCGCATGGACGGCCTACGATATGGACATCTGCATCACCATCCAGAACGCCT
  D  G  G  A  W  T  A  Y  D  M  D  I  C  I  T  I  Q  N  A  Y

       910                       930                       950
ACGAGAAGCAGCACCCGTGGCTCGACCTCTCATCGCTAGGCTTCTGCTACCTCATCTACT
  E  K  Q  H  P  W  L  D  L  S  S  L  G  F  C  Y  L  I  Y  F
```

FIG. 2B

```
          970                       990                      1010
TCAACAGCATGTCGCAGATGARCCGCCAGACGCGCCGGCGCCGCCGTCTGCGCCGCCGCC
  N  S  M  S  Q  M  X  R  Q  T  R  R  R  R  R  L  R  R  R  L

         1030                      1050                      1070
TGGACCTCGCCTACCCGCTCACCGTGGGCTCCATCCCTAAGTCGCAGTCGTGGCCCGTGG
  D  L  A  Y  P  L  T  V  G  S  I  P  K  S  Q  S  W  P  V  G

         1090                      1110                      1130
GTGBCAGCTCGGGHCAGCCCTGCTCCTGMCAGCAGTGCCTGYTGGTCAACAGCACGCGCG
  X  S  S  G  Q  P  C  S  X  Q  Q  C  L  L  V  N  S  T  R  A

         1150                      1170                      1190
CCGTCTCCAACGTCATCCTGGYCTCGCAGCGTCGTAAGGTGMCCCCCGCGCCCCCGCTGT
  V  S  N  V  I  L  X  S  Q  R  R  K  V  X  P  A  P  P  L  S

         1210                      1230                      1250
CGYCGCCGYCGMCACCTGGAGGGCCTCCAGGCGCGCTTGGCGTGCGCCCCAGYGTCACCT
  X  P  X  X  P  G  G  P  P  G  A  L  G  V  R  P  S  V  T  F

         1270                      1290                      1310
TCACAGGCGNCGNGCTCTGNGAAGTGNNNTTCNACGGTCCCGTCGAGCCCGMGYCGTCTC
  T  G  X  X  L  X  E  V  X  F  X  G  P  V  E  P  X  X  S  P

         1330                      1350                      1370
CCGGGGYGCCCCCACGGAGCCCGGGCGCCCCCGGCGGAGCGCGCACCCCGGGGCAGAACA
  G  X  P  P  R  S  P  G  A  P  G  G  A  R  T  P  G  Q  N  N
```

FIG. 2C

```
      1390                1410                1430
ACCTCAACCGGBCCGGGCCCCAGCGCACCACCAGHGTGAGCGCGCGCGCCTCCATCCCGC
  L  N  R  X  G  P  Q  R  T  T  X  V  S  A  R  A  S  I  P  P

      1450                1470                1490
CGGGGGTCCCCGCACTCCCGGTGAAGAACTTGAATGGTACTGGGCCGGTCCATCCGGCCC
  G  V  P  A  L  P  V  K  N  L  N  G  T  G  P  V  H  P  A  L

      1510                1530                1550
TGGCAGGGATGACCGGGATACTGCTGTGCGCGGCCGGGCTGCCCGTGTGCCTGACGCGGG
  A  G  M  T  G  I  L  L  C  A  A  G  L  P  V  C  L  T  R  A

      1570                1590                1610
CCCCCAAGCCCATCCTGCACCCGCCGCCCGTGAGCAAGAGCGACGTGAAGCCCGTGCCTG
  P  K  P  I  L  H  P  P  P  V  S  K  S  D  V  K  P  V  P  G

      1630                1650                1670
GCGTGCCCGGGGTGTGCCGCAAGACCAAGAAGAAGCACCTTAAAAAAGAGTAAGAATCCCG
  V  P  G  V  C  R  K  T  K  K  K  H  L  K  K  S  K  N  P  E

      1690                1710          *     1730     hdx-1
AGGATGTGGTTCGAAGATACATGCAGAAGGTGAAAAACCCACCTGATGAGGACTGCACCA
  D  V  V  R  R  Y  M  Q  K  V  K  N  P  P  D  E  D  C  T  I

      1750                1770                1790
TCTGCATGGAGCGACTGGTCACAGCATCAGGCTACGAGGGCGTGCTTCGGCACAAGGGCG
  C  M  E  R  L  V  T  A  S  G  Y  E  G  V  L  R  H  K  G  V
```

FIG. 2D

```
      1810                  1830  hdx-2           1850
TGCGGCCTGAGCTCGTGGGCCGCCTGGGCCGCTGTGGCCACATGTACCACCTGCTGTGCC
  R  P  E  L  V  G  R  L  G  R  C  G  H  M  Y  H  L  L  C  L

      1870                  1890                  1910
TCGTGGCCATGTACTCCAATGGCAACAAGGATGGCAGCCTGCAGTGCCCCACCTGCAAGG
  V  A  M  Y  S  N  G  N  K  D  G  S  L  Q  C  P  T  C  K  A

      1930 hdx-3            1950        hdx-4     1970
CCATCTACGGGGAGAAGACGGGTACGCAGCCGCCTGGGAAGATGGAGTTCCACCTCATCC
  I  Y  G  E  K  T  G  T  Q  P  P  G  K  M  E  F  H  L  I  P

      1990                  2010                  2030  $
CCCACTCGCTGCCCGGCTTCCCTGATACCCAGACCATCCGCATCGTCTATGACATCCCCA
  H  S  L  P  G  F  P  D  T  Q  T  I  R  I  V  Y  D  I  P  T

      2050                  2070                  2090
CAGGCATCCAGGGCCCTGAGCACCCCAACCCCGGGAAGAAGTTCACCGCAAGAGGATTCC
  G  I  Q  G  P  E  H  P  N  P  G  K  K  F  T  A  R  G  F  P

      2110                  2130                  2150
CTCGCCACTGCTATCTACCCAACAACGAGAAAGGCCGGAAGGTGCTGCGGCTGCTCATCA
  R  H  C  Y  L  P  N  N  E  K  G  R  K  V  L  R  L  L  I  T

      2170                  2190                  2210
CGGCCTGGGAGAGAAGACTCATCTTCACTATCGGCACGTCCAACACCACGGGCGAGTCGG
  A  W  E  R  R  L  I  F  T  I  G  T  S  N  T  T  G  E  S  D
```

FIG. 2E

```
      2230                  2250                  2270
ACACCGTGGTGTGGAACGAGATCCACCACAAGACCGAGTTTGGATCCAACCTCACGGGAC
  T  V  V  W  N  E  I  H  H  K  T  E  F  G  S  N  L  T  G  H

      2290                  2310                  2330
ACGGCTACCCGGACGCTAGCTACCTAGACAACGTGCTGGCTGAGCTCACAGSCCAGGGCG
  G  Y  P  D  A  S  Y  L  D  N  V  L  A  E  L  T  X  Q  G  V

      2350                  2370                  2390
TATCCGAGGCTGCAGGCAAGGCTTGAGGSCCAAGGCTGCCCACCTTCCCTCCTGSTTTGG
  S  E  A  A  G  K  A  END

      2410                  2430                  2450
CCCTGGTCCGGCAAATGCcTCCTTCGCCAGGTGTGTCCTGGTAGCCCAGGTTCAGGGCTG

      2470                  2490                  2510
GGGAGGAGCCTGCGGAAGGGGCCGCAGCCATTCAGGGGACTGNCTGGNGGAAGTTGGATG

      2530
AGGAGAGNTGGATTTNAGGTTGGCCCC
```

FIG. 2F

```
1    MASSAGSAASGSVVPGGGGSAASSCATMALSTAGSGGPPV   FLY DELTEX
1    MSR----------PGHGG----------LMPVNGLGFPPQ   HUMAN DELTEX

41   NHAHAVCVWE-FESRGKWLPYSPAVSQHLERA-HAKKLTR   FLY DELTEX
21   NVARVV-VWECLNEHSRWRPYTATVCHHIENVLKEDARGS   HUMAN DELTEX

79   VMLSDADPSLEQYYVNVRTMTQESEAETRSGLLTIGVRRM   FLY DELTEX
60   VVLGQVDAQLVPYIIDLQSMHQFRQD---TGTMR-PVRRN   HUMAN DELTEX

119  LYAPSSPAGKGTKWEWSGGSADSNNDWRPYNMHVQCI-IE   FLY DELTEX
96   FYDPSSAPGKGIVWEWENDGGA----WTAYDMDI-CITIQ   HUMAN DELTEX

158  DAWARGEQTLDLCNTHIGLPYTINFCNLTHVRQPSGPMRS   FLY DELTEX
131  NAYEKQHPWLDLSS--LGFCYLIYFNSMSQMXRQTRRRRR   HUMAN DELTEX

198  IRRTQQAPYPLV--KLTPQQANQLKSNSASVSSQYNTLPK   FLY DELTEX
169  LRRRLDLAYPLTVGSIPKSQSWPVGXSSGQPCSXQQCLL-   HUMANDELTEX

236  LGDTKSLHRVPMTRQQHPLPTSHQVQQQQHQLQHQQQQQQ   FLY DELTEX
208  VNSTRAVSNVILXSQRRKVXPAPPLSXPXX----------   HUMAN DELTEX

276  QHHHQHQQQQHQQQQQHQMQHHQIHHQTAPRKPPKKHSEI   FLY DELTEX
238  ----------------------------PGGPPGALGVR   HUMAN DELTEX
```

FIG.3A

```
316 S T T N L R Q I L N N L N I F S S S T K H Q S N M S T A A S A S S S S S A S L   FLY DELTEX
249 P S V T F T G X X L X E V X F X G P V E P X X S P G X P P R S P G A P G G A R T   HUMAN DELTEX

356 H H A N H L S H A H F S H A K N M L T A S M N S H H S R C S E G S L Q S Q R S S   FLY DELTEX
289 P G Q N N L N R X - - - - - - - - - - - - - - - - - - - - - - - - G P Q R T T   HUMAN DELTEX

396 R M G S H R S R S R T R T S D T D T N S V K S H R R R P S V D T V S T Y L S H E   FLY DELTEX
304 X V S A R A S I P P G V P A L P V K N L N G T G P V H P A L A G M T G I L - - -   HUMAN DELTEX

436 S K E S L R S R N F A I S V N D L L D C S L G S D E V F V P S V P P S S L G E R   FLY DELTEX
341 - - - - - - - - - - - - L C A A G L P V C - L T R A P K P I L H P P P V S K S D V   HUMAN DELTEX

476 A P V P P P L P L H P R Q Q Q Q Q Q Q Q Q L Q M Q Q Q Q Q A Q Q Q Q Q Q S I   FLY DELTEX
369 K P V P G V P G V C R K T K K K H L K K S K N - - - - - - - - - - - - - - - - - -   HUMAN DELTEX

516 A G S I V G V D P A S D M I S R F V K V V E P P L W P N A Q P C P M C M E E L V   FLY DELTEX
392 - - - - - - - - - - P E D V V R R Y M Q K V K N - - - P P D E D C T I C M E R L V   HUMAN DELTEX

556 - - - - - - - - - - - H S A Q N P A I - - S L S R C Q H L M H L Q C L N G M I I A   FLY DELTEX
420 T A S G Y E G V L R H K G V R P E L V G R L G R C G H M Y H L L C L V A M Y - -   HUMAN DELTEX

584 Q Q N E M N K N L F I E C P V C G I V Y G E K V G N Q P I G S M S W S I I S K N   FLY DELTEX
458 - - S N G N K D G S L Q C P T C K A I Y G E K T G T X P P G K M E F H L I P H S   HUMAN DELTEX
```

FIG.3B

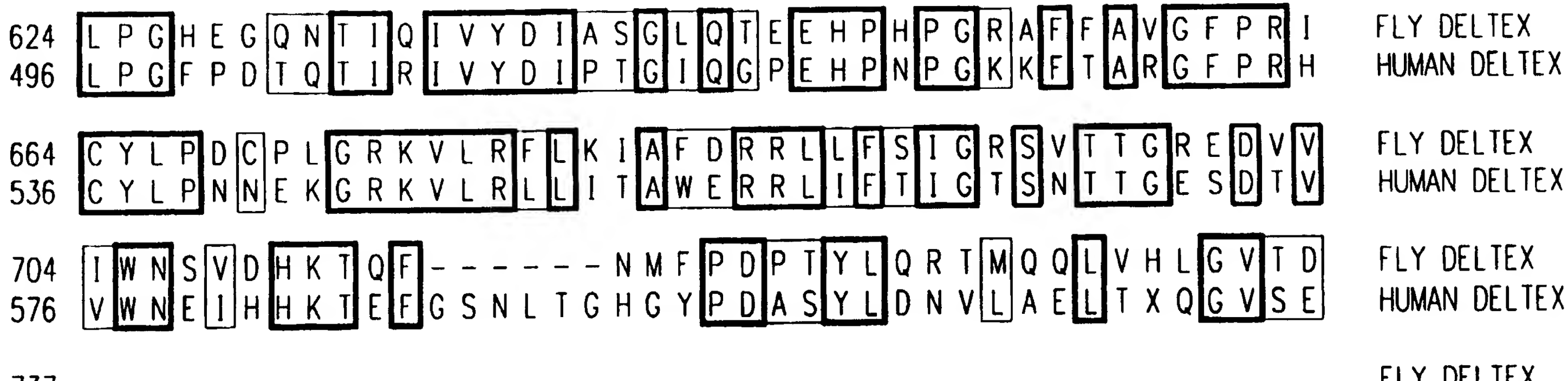

FIG.3C

```
         10         20         30         40         50         60
         *    *     *    *     *    *     *    *     *    *     *
MASSAGSAAS GSVVPGGGGS AASSCATMAL STAGSGGPPV NHAHAVCVWE FESRGKWLPY
                                  A                               >
                                  D                               >

         70         80         90         100        110        120
         *    *     *    *     *    *     *    *     *    *     *
SPAVSQHLER AHAKKLTRVM LSDADPSLEQ YYVNVRTMTQ ESEAETRSGL LTIGVRRMLY
                                  A                               >
                                  D                               >

        130        140        150        160        170        180
         *    *     *    *     *    *     *    *     *    *     *
APSSPAGKGT KWEWSGGSAD SNNDWRPYNM HVQCIIEDAW ARGEQTLDLC NTHIGLPYTI
                                  A                               >
                                  D                               >
```

FIG. 4A

```
      190       200       210       220       230       240
        *    *    *    *    *    *    *    *    *    *
NFCNLTHVRQ PSGPMRSIRR TQQAPYPLVK LTPQQANQLK SNSASVSSQY NTLPKLGDTK
_________________________________A_______________________________>
__________D___________>

      250       260       270       280       290       300
   *    *    *    *    *    *    *    *    *    *    *
SLHRVPMTRQ QHPLPTSHQV QQQQHQLQHQ QQQQQQHHHQ HQQQQHQQQQ QHQMQHHQIH
_________________________________A_______________________________>

      310       320       330       340       350       360
        *    *    *    *    *    *    *    *    *    *    *
HQTAPRKPPK KHSEISTTNL RQILNNLNIF SSSTKHQSNM STAASASSSS SSASLHHANH
___>
     _______________________________B____________________________>
```

FIG. 4B

```
      370         380         390         400         410         420
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
LSHAHFSHAK NMLTASMNSH HSRCSEGSLQ SQRSSRMGSH RSRSRTRTSD TDTNSVKSHR
                                        B                          >

      430         440         450         460         470         480
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
RRPSVDTVST YLSHESKESL RSRNFAISVN DLLDCSLGSD EVFVPSVPPS SLGERAPVPP
                                        B                          >

      490         500         510         520         530         540
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
PLPLHPRQQQ QQQQQQQQLQ MQQQQQAQQQ QQQSIAGSIV GVDPASDMIS RFVKVVEPPL
          >                                                        C
                                                                   >

      550         560         570         580         590         600
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
WPNAQPCPMC MEELVHSAQN PAISLSRCQH LMHLQCLNGM IIAQQNEMNK NLFIECPVCG
                                        C                          >
```

FIG. 4C

610 620 630 640 650 660

IVYGEKVGNQ PIGSMSWSII SKNLPGHEGQ NTIQIVYDIA SGLQTEEHPH PGRAFFAVGF

C

670 680 690 700 710 720

PRICYLPDCP LGRKVLRFLK IAFDRRLLFS IGRSVTTGRE DVVIWNSVDH KTQFNMFPDP

C

730

TYLQRTMQQL VHLGVTD

C

FIG. 4D

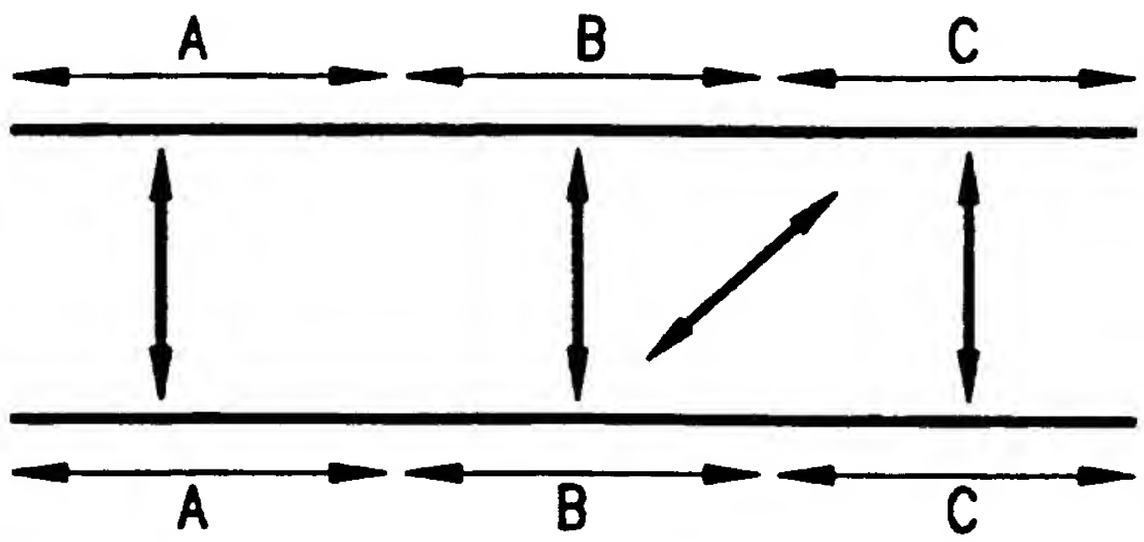

FIG.5

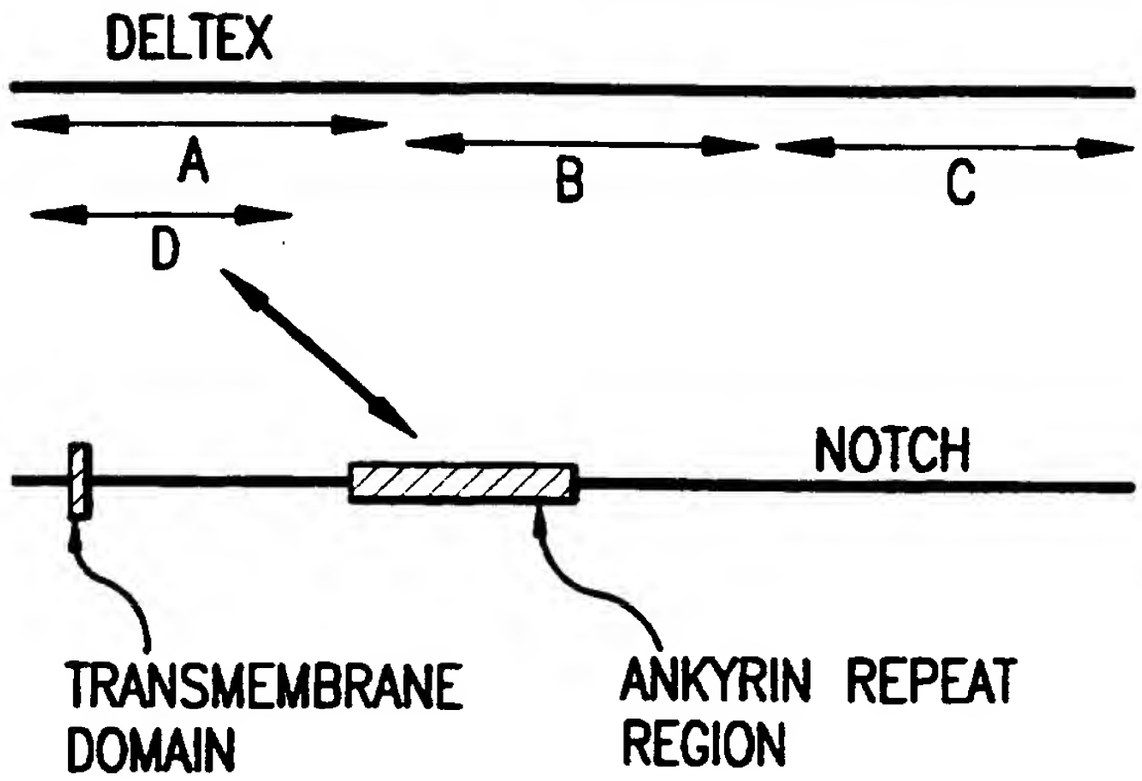

FIG.6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18675

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data bases.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, GENBANK, MEDLINE, EMBASE, BIOSIS.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	XU, Tian et al. deltex, a Locus Interacting with the Neurogenic Genes, Notch, Delta and mastermind in Drosophila melanogaster. Genetics. November 1990, Vol. 126, pages 665-677, see entire document.	1-91
Y	BLANK, Volker et al. NF-κB and related proteins: Rel/dorsal homologies meet ankyrin-like repeats. Trends in Biochemical Sciences. April 1992, Vol 17, pages 135-140, see entire document.	1-91
Y	ARTAVANIS-TSAKONAS, Spyros et al. Choosing a cell fate: a view from the Notch locus. Trends in Genetics. November/December 1991, Vol. 7, No. 11/12, pages 403-408, see entire document.	1-91

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
12 MARCH 1997	17 APR 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ERNEST JONES JAB for Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18675

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92/19734 A1 (YALE UNIVERSITY) 12 November 1992, see entire document.	1-91
Y	WO 95/19779 A1 (YALE UNIVERSITY) , 27 July 1995, see entire document.	1-91

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18675

Box I Observations where certain claims were found unsearchable (C ntinuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18675

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 35/12; C07H 17/00; C07K 1/00, 14/00, 16/00; C12N 1/00, 5/00, 15/00; C12Q 1/00, 1/68; G01N 33/53

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/130.1; 435/6, 7.1, 69.1, 172.1, 172.3, 243, 320.1, 325; 514/2, 44; 530/350, 387.1, 388.1; 536/23.1, 23.2, 23.4, 23.5, 24.1, 24.3

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

424/130.1; 435/6, 7.1, 69.1, 172.1, 172.3, 243, 320.1, 325; 514/2, 44; 530/350, 387.1, 388.1; 536/23.1, 23.2, 23.4, 23.5, 24.1, 24.3

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

I. Claims 1-12, 17-20, and 87-91 drawn to purified Deltex proteins.
II. Claims 13-16, drawn to chimeric proteins comprising a portion of Deltex proteins.
III. Claims 21-24, drawn to antibodies to Deltex proteins.
IV. Claims 25-46, 48-54, drawn to nucleic acids encoding Deltex proteins and associated vectors and host cells.
V. Claim 47, drawn to nucleic acids encoding chimeric proteins.
VI. Claims 55-62, 66-72, 74-76, 78-80, drawn to pharmaceutical compositions comprising Deltex proteins and methods of treating a disease using said compositions (Note that claims 66-72, and 78 will be examined only to the extent that they read on the use of Deltex proteins as therapeutic agents.)
VII. Claims 63-64, 66-72, 78, and 81, drawn to pharmaceutical compositions comprising Deltex protein encoding nucleic acids and methods of treating a disease using said compositions. (Note that claims 66-72, and 78 will be examined only to the extent that they read on the use of Deltex protein encoding nucleic acids as therapeutic agents.)
VIII Claims 65,66-73, 78 and 82, drawn to pharmaceutical compositions comprising antibodies to Deltex proteins and methods of treating a disease using said compositions.(Note that claims 66-72, and 78 will be examined only to the extent that they read on the use of antibodies to Deltex protein as therapeutic agents.)
IX. Claims 66-72, 77-78, 83-86, drawn to drawn to pharmaceutical compositions comprising Deltex antisense nucleic acids and methods of treating a disease using said compositions. (Note that claims 66-72, and 78 will be examined only to the extent that they read on the use of Deltex antisense nucleic acids as therapeutic agents.)

The compositions of the inventions of groups I - III do not share any special technical feature because they are drawn to divergent chemical compositions with differing properties. For example the invention of groups I and II are drawn to proteins and that of group III to nucleic acids. Aside from their divergent chemical natures, these groups of compositions require separate analysis. For example, consideration of nucleic acids requires analysis of gene coding regions and expression vectors and considerations of proteins require analysis of protein isolation purification and function. The antibodies of the invention of group III are distinct from the proteins of group I because antibody compositions require analysis of means of producing specific protein binding reagents and uses thereof and such analysis is not required for consideration of structural proteins per se. Similarly, the inventions of groups IV-VI do not share a special technical feature because they are based upon the use of materially different compositions; proteins, nucleic acids and antibodies, respectively.

The proteins of the invention of group I do not share a special technical feature with the pharmaceutical compositions of the invention of group IV because said proteins may be used for the in vitro production of antibodies which does not involve use in the in vivo environment. Therefore, analysis of the proteins of group I does not require consideration of means of administration and assays for determination of efficacy as required for the analysis of the invention of group

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18675

IV. Similarly, the antibodies of the invention of group II do not share a special technical feature with the pharmaceutical compositions of the invention of group VI because the latter invention requires analysis of the in vivo introduction of antibodies and because the antibodies of the invention of group II may be used in vivo.

The proteins of the invention of group I do not share a special technical feature with any of the methods of groups V-VII because said methods do not utilize said proteins and therefore consideration of said proteins per se is not required for analysis of any of said groups of methods.

The proteins of the invention of group I do not share a special technical feature with the method of the invention of group VII because said method requires consideration of disease states and correlations between protein binding and said states and such considerations and analysis is not required for examination of proteins per se.

The antibodies of the invention of group III do not share a special technical feature with any of the methods of groups IV, V, VII, and VIII because said methods of not utilize said antibodies and therefore, consideration of said antibodies per se is not required for analysis of any of said groups and methods.

The nucleic acids of the invention of group V do not share a special technical feature with the pharmaceutical compositions of the invention of groups IV, and VI because said compositions require consideration of in vivo use (see above) which is not required for analysis of nucleic acids per se and because said nucleic acids may be used in vitro. For example, said nucleic acids may be use to produce proteins.

The nucleic acids of the invention of group IV do not share a special technical feature with the method of group VIII because said method does not utilize said nucleic acids and therefore consideration of said nucleic acids per se is not required for the analysis of said method.

The nucleic acids of the invention of group IV do not share a special technical feature with the methods and compositions of the invention of group IX because the latter invention involves the use of antisense nucleotides which do not encode proteins, which regulate gene expression and which are designed for in vivo use. In contrast, the nucleic acids of the invention of group IV encodes proteins (non-regulatory) and may be used for in vitro production of proteins. Therefore, analysis of the nucleic acids of the invention of group IV does not require the in vivo or regulatory considerations required for analysis of the invention of group VII. Similarly, the inventions of group V and IX do not share a special technical feature because the former invention utilizes nucleic acids encoding proteins and the latter invention utilizes antisense nucleic acids that regulate gene expression.

The invention of group VI does not share a special technical feature with the methods of the invention of group IX because the latter methods utilize antisense nucleic acid therapeutics whereas the former utilize proteins. Consideration of the use of antisense nucleic acids requires analysis of selection of appropriate sequences and means of regulating gene expression and such analysis is not required for consideration of methods based upon protein therapeutic agents.

The invention of any of groups IV-VII does not share a special technical feature with the methods of group VIII because the former inventions utilize pharmaceutical compositions for in vivo treatment whereas the latter invention uses proteins for in vitro diagnostic. Therefore, the method of group VIII does not require considerations of in vivo methodologies.

The invention of group VI does not share a special technical feature with the invention of group VII because the former invention utilizes proteins and the latter invention utilizes nucleic acids. Therefore, the analysis of each invention requires considerations distinct to each distinct class of pharmaceutical.

Therefore, the separate inventions as listed above are not so linked by any single special technical feature so as to form a single inventive concept within the meaning of PCT Rule 13.2. It is noted that the Deltex proteins are used in all of the inventions of groups I-IV and VI-VIII, however, these proteins were known in the prior art and therefore do not represent a contribution over the prior art as defined by PCT Rule 13.2.